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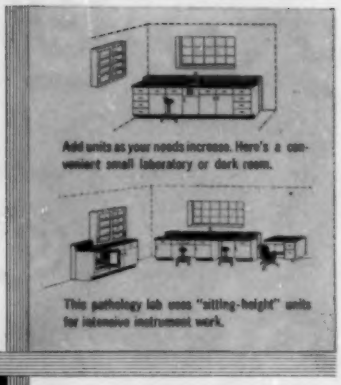

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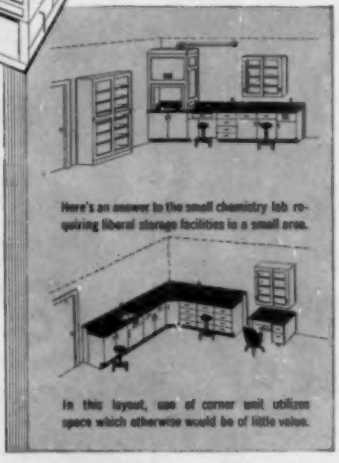

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FINAL NATIONAL FORMULARY ADMISSIONS AND DELETIONS

The tenth edition of the National Formulary is now in the final stage of preparation for release, with publication anticipated within the next three or four months. During the course of the revision of N. F. IX, preparatory to the publication of N. F. X, many significant changes occurred, and those involving additions and deletions have now been tabulated in final form.

Table I constitutes a list of titles for 259 new monographs admitted to N. F. X. During the revision of N. F. IX, a total of 241 monographs included therein were not admitted to N. F. X, and these are listed in Table II. The reasons for the individual deletions vary, but in general the N. F. IX monographs not admitted to N. F. X were dropped because they had become practically obsolete. About forty deletions, however, represent admissions to U. S. P. XV on the basis of either therapeutic essentiality or pharmaceutical necessity. Of the 259 new monographs, 129 in several different therapeutic categories will become official for the first time. The balance of the admissions new to the National Formulary were chosen from those deleted from U. S. P. XIV. In Table I, listing the final new admissions to N. F. X, the items from the U. S. P. XIV list of deletions are indicated by an asterisk. In Table II, N. F. IX drugs admitted to U. S. P. XV are similarly identified.

TABLE I.—LIST OF NEW ADMISSIONS TO N. F. X.

A

Acetanilid*
Acetanilid Tablets*
Acetylsalicylic Acid Capsules*
Acetylsalicylic Acid, Acetophenetidin and Caffeine Capsules
Acetylsalicylic Acid, Acetophenetidin and Caffeine Tablets
Allylbarbituric Acid
Allylbarbituric Acid Tablets
Aloin*
Aminopyrine*
Aminopyrine Tablets*
Amphetamine Phosphate
Amphetamine Phosphate Injection
Amphetamine Phosphate Tablets
Amphetamine Phosphate, Dibasic
Amphetamine Phosphate, Dibasic, Tablets
d-Amphetamine Phosphate
d-Amphetamine Phosphate Tablets
d-Amphetamine Phosphate, Dibasic
d-Amphetamine Phosphate, Dibasic, Tablets
Aprobarbital
Atropine*
Aurothioglucose
Aurothioglucose Injection

B

Barbital*
Barbital Tablets*
Barbital Sodium*
Barbital Sodium Tablets*
Belladonna Extract*
Bishydroxycoumarin Capsules*
Bismuth Potassium Tartrate*
Bismuth Potassium Tartrate Injection*
Boric Acid Ointment*
Bromisovalum
Bromisovalum Tablets
Butacaine Sulfate*
Butallylonal
Butallylonal Tablets
Butethal
Butethal Tablets
Butethamine Hydrochloride
Butethamine Hydrochloride and Epinephrine Injection
Butyl Chloride

C

Calcium Lactate*
Calcium Lactate Tablets*
Camphor Liniment*
Camphor Water*
Caraway*
Carbol-Fuchsin Solution
Cardamom Seed*
Cardamom Tincture, Compound*
Cascara Sagrada Extract*
Cascara Sagrada Extract Tablets*
Cascara Sagrada Fluidextract*
Chloroazodin*
Chloroazodin Solution*
Choline Bitartrate
Choline Bitartrate Capsules
Choline Bitartrate Tablets
Choline Dihydrogen Citrate
Choline Dihydrogen Citrate Capsules
Choline Dihydrogen Citrate Tablets
Cinnamon*
Cobalamin Concentrate
Cocaine*
Cochineal*
Codeine Sulfate*
Codeine Sulfate Tablets*
Cupric Sulfate*
Cyclobarbital
Cyclobarbital Tablets

D

Danthron
Diallylbarbituric Acid
Diallylbarbituric Acid Tablets
Diethylstilbestrol Injection*
Digitalis Injection*
Digitalis Tincture*
Dihydrocodeinone Bitartrate
Dihydrocodeinone Bitartrate Syrup
Dihydrocodeinone Bitartrate Tablets
Dihydroxyaluminum Aminoacetate
Dihydroxyaluminum Aminoacetate Magma
Dihydroxyaluminum Aminoacetate Tablets
Dimethyl Tubocurarine Iodide
Dimethyl Tubocurarine Iodide Injection
Diphtheria and Tetanus Toxoids*
Diphtheria Toxoid and Pertussis Vaccine Combined*
Drocarbil

E

Ephedrine*
Ephedrine Hydrochloride*
Ephedrine Hydrochloride Capsules*
Ephedrine Hydrochloride Tablets*
Epinephrine Bitartrate Ophthalmic Solution
Erythrityl Tetranitrate Tablets*
Estradiol*
Estradiol Injection, Aqueous
Estradiol Pellets
Estradiol Tablets*
Ethyl Aminobenzoate*
Ethyl Vanillin

F

Ferric Ammonium Citrate*
Ferric Ammonium Citrate Capsules*
Fuchsin, Basic

G

Gentian*
Gentian Tincture, Compound*
Glyceryl Triacetate*
Glycobiarsol

* Items admitted to N. F. X. from U.S.P. XIV

(*Continued on page IV*)

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TABLE I (*Continued from page II*)

Glycobiarsol Tablets
Glycyrrhiza Extract*
Gold Sodium Thiomalate
Gold Sodium Thiomalate Injection

H

Hexavitamin Capsules*
Hexavitamin Tablets*
Hexestrol
Hexestrol Injection
Hexestrol Tablets
Hexobarbital Sodium
Hexobarbital Sodium, Sterile
Hydriodic Acid, Diluted*
Hydriodic Acid Syrup*
Hydrochloric Acid, Diluted*
Hyoscyamine Hydrobromide
Hyoscyamine Sulfate
Hypophosphorous Acid*

I

Inositol
Inositol Tablets
Iodine Solution, Phenolated

K

Kaolin Mixture with Pectin

L

Lanatoside C*
Lanatoside C Tablets*
Lidocaine
Lidocaine Hydrochloride Injection

M

Magnesium Citrate Solution*
Mannitol
Mannitol Injection
Mechlorethamine Hydrochloride, Sterile
Mechlorethamine Hydrochloride Trituration, Sterile
Menadione Injection*
Mephenesin
Mephenesin Capsules
Mephenesin Tablets
Mephobarbital
Mephobarbital Tablets
Mercuric Oxide, Yellow*
Mercuric Oxide, Yellow, Ointment*
Mercury Bichloride Ophthalmic Ointment*
Methacholine Chloride Capsules*
Methaphenilene Hydrochloride
Methaphenilene Hydrochloride Tablets
Methionine
Methionine Capsules
Methionine Tablets
Monoethanolamine

N

Naepaine Hydrochloride
Naphazoline Hydrochloride*
Naphazoline Hydrochloride Solution, Mild*
Naphazoline Hydrochloride Solution, Strong*
Neocinchophen*
Neocinchophen Tablets*
Nicotinic Acid Capsules*
Nitrofurazone
Nitrofurazone Ointment
Nitrofurazone Solution

O

Octylphenoxy Polyethoxyethanol
Oleovitamin A and D*
Oleovitamin A and D Capsules
Orange Flower Water*
Orange Peel, Bitter*
Ox Bile Extract*
Ox Bile Extract Capsules*
Ox Bile Extract Tablets*

P

Pancreatin*
Para-aminobenzoic Acid*
Para-aminobenzoic Acid Tablets*
Para-aminohippuric Acid
Para-nitrosulfathiazole
Para-nitrosulfathiazole Suspension
Penicillin for Inhalation*
Penicillin Ointment*
Penicillin Procaine in Oil Injection*
Penicillin Troches*
Pentobarbital Sodium Injection*
Pentobarbital Sodium Tablets*
Petrolatum, Liquid, Emulsion*
Petroleum Benzin*
Pheniramine Maleate
Pheniramine Maleate Solution, Ophthalmic
Pheniramine Maleate Tablets
Phenobarbital Sodium Injection*
Phenobarbital Sodium Tablets*
Phenylethyl Alcohol
Phthalylsulfacetamide
Phthalylsulfacetamide Tablets
Picrotoxin*
Picrotoxin Injection*
Polyethylene Glycol 300
Polyethylene Glycol 1540
Potassium Bromide*
Potassium Nitrate*
Potassium Phosphate
Probarbital Sodium
Probarbital Sodium Tablets
Promethazine Hydrochloride
Promethazine Hydrochloride Tablets
Protamine Sulfate Injection

Q

Quinidine Gluconate
Quinidine Gluconate Injection
Quinine Dihydrochloride*

R

Rosemary Oil*

S

Sarsaparilla*
Sarsaparilla Fluidextract*
Sarsaparilla Syrup, Compound*
Sassafras Oil*
Scopolamine Hydrobromide Tablets*
Sodium Para-aminohippurate Injection
Sodium Phosphate*
Sodium Phosphate, Effervescent*
Sodium Phosphate, Exsiccated*
Sodium Psylliate Injection
Sodium Salicylate Injection*
Sodium Sulfate*
Sorbitol Solution
Spearment Oil*
Sulfacetamide, Sulfadiazine, and Sulfamerazine Suspension
Sulfacetamide, Sulfadiazine, and Sulfamerazine Tablets
Sulfadiazine and Sulfamerazine Tablets
Sulfaguanidine*
Sulfaguanidine Tablets*
Sulfamerzine Sodium Injection*
Sulfanilamide*
Sulfanilamide Tablets*
Sulfathiazole*
Sulfathiazole Tablets*
Sulfathiazole Sodium*
Sulfathiazole Sodium Injection*
Sulfathiazole Sodium, Sterile*

T

Tartaric Acid*
Theobromine Calcium Salicylate*
Theobromine Calcium Salicylate Tablets*
Theobromine Sodium Acetate*
Theobromine Sodium Acetate Capsules*
Theobromine Sodium Acetate Tablets*
Theophylline Sodium Glycinate
Theophylline Sodium Glycinate Tablets
Thimerosal Solution
Thimerosal Tincture
dl-alpha-Tocopherol
Tocopherols, Mixed, Concentrate

(*Continued on page V*)

TABLE I (*Continued form page IV*)

d-alpha-Tocopheryl Acetate
d-alpha-Tocopheryl Acetate Concentrate
dl-alpha-Tocopheryl Acetate
Triasyn B Capsules*
Triasyn B Tablets*
Tuaminoheptane Sulfate
Tyrothricin Solution*
Tyrothricin Troches*

V

Vinbarbital
Vinbarbital Capsules
Vinbarbital Sodium Injection

Z

Zinc Compounds and Eugenol Cement

TABLE II.—N. F. IX ARTICLES NOT ADMITTED TO N. F. X.

A

Acetanilid Powder, Compound
Aconite Fluidextract
Aloe Pills
Aloe Tincture
Aloin, Strychnine and Belladonna Pills
Aloin, Strychnine, Belladonna and Cascara Pills
Aloin, Strychnine, Belladonna and Ipecac Pills
Althea Syrup
Aluminum Chloride*
Aluminum Chloride Solution
Ammonia Liniment
Ammonia Spirit, Anisated
Ammonium Bromide Elixir
Ammonium Valerate, Acid
Amobarbital*
Amobarbital Tablets*
Amobarbital Sodium*
Amobarbital Sodium Capsules*
Amobarbital Sodium, Sterile*
Anethole*
Anise
Anise Spirit
Anise Water
Arnica Fluidextract
Aromatic Powder
Aromatic Spray
Arsenious Acid Solution

B

Barbital Elixir
Barium Chloride
Belladonna Liniment
Benzaldehyde Spirit
Benzoic and Salicylic Acid Ointment*
Birch Tar Oil, Rectified
Bismuth Subgallate Tablets
Brucine Sulfate

C

Calamine Liniment
Calamine Lotion, Phenolated*
Calcium and Sodium Glycerophosphates Elixir
Camphor Injection
Camphor Ointment
Capsicum Ointment
Carbon Tetrachloride Capsules
Cardamom Elixir, Compound
Carmine
Carmine Solution
Cerium Oxalate
Chalk Powder, Aromatic
Charcoal, Purified Animal
Chaulmoogra Oil
Chenopodium Oil Capsules
Cholera Vaccine*
Cinchona
Cinchona Alkaloids Elixir
Cinchona Tincture, Compound
Cinchonidine Sulfate
Cinchonine Sulfate
Cinnamaldehyde
Cinnamon Spirit
Cinnamon Syrup
Cinnamon Tincture
Coal Tar Solution*
Coal Tar Solution, Chloroformic
Cochineal Solution
Coconut Oil
Colchicum Corm
Colchicum Corm Fluidextract
Colchicum Corm Tincture, Strong
Collodion, Salicylic*
Colocynth and Jalap Pills, Compound
Copaiba
Creosote Carbonate
Cubeb Oleoresin

D

Dehydrocholic Acid*
Dehydrocholic Acid Tablets*
Dibucaine Hydrochloride*
Digitalis Extract
Digitalis Infusion
Dioctyl Sodium Sulfosuccinate*

E

Ergot Extract
Ergot, Prepared
Ether Spirit
Ether Spirit, Compound
Ethereal Oil

F

Ferric Ammonium Citrate, Green, Injection
Ferric Cacodylate Injection
Ferric Oxide, Red
Ferric Oxide, Yellow
Ferrous Gluconate*
Formaldehyde Solution*
Formic Acid
Formic Acid Spirit
Frangula

G

Gelsemium
Gelsemium Fluidextract
Gelsemium Tincture
Gentian Elixir
Gentian Extract
Ginger Syrup
Glyceryl Trinitrate Spirit
Guaiac Tincture, Ammoniated

H

Hamamelis Leaf
Hamamelis Leaf Fluidextract
Hydrastis Tincture
Hydroxystearin Sulfate
Hyoscyamus Fluidextract
Hypophosphites Syrup

I

Iodides Tincture
Iodized Ointment, Stainless
Ipecac Tincture
Iron, Peptonized
Iron, Peptonized, and Manganese Solution
Iron, Reduced, Capsules

J

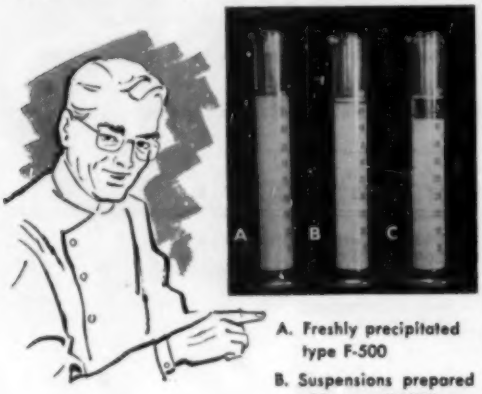
Juniper Oil

K

Kaolin Cataplasm

(*Continued on page VI*)

just what the doctor ordered



A. Freshly precipitated type F-500
B. Suspensions prepared with type F-1000
C. Suspensions prepared with type F-2000

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TABLE II (*Continued from page V*)

L

Larkspur
Larkspur Tincture
Larkspur Tincture, Acetic
Lavender Spirit
Lead and Opium Lotion
Leptandra
Leptandra Extract
Lobelia Fluidextract

Mandelic Acid
Menthol Spray, Compound
Mercuric Oxide, Red
Mercuric Oxide, Red, Ointment
Mercuric Salicylate
Mercuric Salicylate Injection
Mercuric Succinimide
Mercuric Succinimide Injection
Mercury Mass
Methylcellulose*
Methylrosaniline Chloride*
Methylrosaniline Chloride Jelly
Methylrosaniline Chloride Solution*
Morphine Hydrochloride*

N

Neoarsphenamine
Neocalamine Liniment
Neocalamine Lotion
Neocalamine Lotion, Phenolated
Neocalamine Ointment
Neocalamine, Prepared
Nitrohydrochloric Acid
Nitrohydrochloric Acid, Diluted
Nutgall
Nutgall Ointment

O

Oleyl Alcohol
Opium Extract

P

Pamaquine Naphthoate
Pectin Paste
Pectin Paste, Thin
Pepsin Elixir
Pepsin, Saccharated
Petroxolin, Solid
Phenylmercuric Chloride
Pine Oil Emulsion Concentrate
Plague Vaccine
Podophyllum*
Podophyllum Resin*
Potash, Sulfurated*
Potassium Bromide Elixir
Potassium Chlorate Gargle with Iron
Potassium Chlorate Tablets
Potassium Citrate, Effervescent
Potassium Citrate Solution
Potassium Guaiacolsulfonate Syrup
Potassium Permanganate Tablets*
Procaine Borate
Proflavine Dihydrochloride
Proflavine Sulfate

Q

Quillaja
Quinine Hydrobromide

R

Resorcin Brown
Resorcin Brown Solution
Rhubarb Elixir, Alkaline
Rhubarb Extract
Rhubarb Powder, Compound
Rhubarb Syrup
Rhubarb Syrup, Aromatic
Rhubarb Tincture
Rhubarb Tincture, Sweet

(*Continued on page VII*)

TABLE II (*Continued from page VI*)

S

Salicin
Santal Oil
Scarlet Fever Streptococcus Antitoxin
Scarlet Fever Streptococcus Toxin
Scarlet Red Sulfonate
Serum, Antimeningococcic
Serum, Antipneumococcic
Serum, Human Measles Immune
Serum, Human Scarlet Fever Immune
Serum, Normal Human
Silver Chloride, Colloidal
Silver Nitrate, Toughened*
Soap, Soft, Liniment*
Soap, Soft, Liniment, Compound
Sodium Carbonate, Monohydrated*
Sodium Chloride Tablets*
Sodium Citrate Solution
Sodium Hydroxide*
Sodium Indigotindisulfonate*
Sodium Indigotindisulfonate Injection*
Sodium Perborate, Monohydrated
Sodium Salicylate Elixir
Sodium Thiocyanate Elixir
Spearmint Spirit
Spearmint Water
Stearic Acid*
Stramonium Extract
Stramonium Fluidextract
Stronitium Salicylate
Strophanthus
Strophanthus Tincture
Strychnine Nitrate Tablets
Succinchlorimide
Succinchlorimide Tablets
Suet, Prepared
Sulfapyridine*
Sulfapyridine Tablets*
Sulfarsphenamine
Sulfur Ointment, Compound
Sun Cream, N. F.

T

Tannic Acid Ointment
Tar, Juniper*
Thimerosal Jelly
Thyme Fluidextract
Thyme Syrup
Totaquine Capsules
Totaquine Tablets
Tragacanth Glycerite
Trinitrophenol
Turpentine

U

Undecylenic Acid*

V

Vanillin Elixir, Compound
Vanillin Spirit, Compound
Veratrum Viride Tincture
Viburnum Prunifolium Elixir
Viburnum Prunifolium Fluidextract

W

White Lotion*
Wintergreen Water

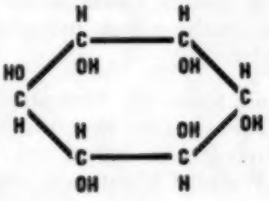
Z

Zinc Oxide Hard Paste
Zinc Oxide Soft Paste
Zinc Undecylenate*

* N. F. IX deletions admitted to U. S. P. XV.

INOSITOL

A Vitamin and Lipotropic Agent



Inositol, of interest in many biological systems is a pure, crystalline, naturally-occurring component of the vitamin-B complex. Its relationships to cholesterol and fat metabolism and its existence in physiologically important phospholipids are evidence of its significance as a nutritional factor.

Inositol is known to exhibit a synergistic action with other lipotropic agents. It is an accessory growth factor for animals and microorganisms.

Literature available upon request.

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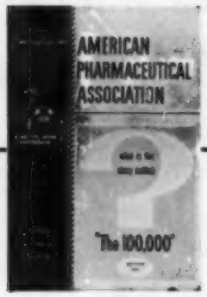


A Preview of Advertising

In the April 1955 issue of the Practical Pharmacy Edition

(All references to actions and uses are taken from the statements of the producers of the products mentioned.)

▶ In a two-page spread, **Abbott Laboratories** features its antibiotic, *filmtab* **Erythrocin Stearate,** *"specific therapy against the cocci with little risk of serious side effects,"* and in another insertion, **SUR-BEX** (B-Complex Tablets with C)—recommended as a dietary supplement and indicated, also, for *stress.*

▶ Much of its renown is attributed by **The American Druggists' Insurance Co.** to the *service* it renders its clients.

▶ With the admonition, "To Keep Up, Keep Stocked," **Bristol Laboratories** advises that wholesalers can make immediate deliveries of **Polycycline** in all four dosage forms—capsules, suspension, pediatric drops and intramuscular.

▶ To meet the demand for a narrow petrolatum gauze strip, **Chesebrough Mfg. Co.** is marketing a new one-inch size **Vaseline** strip—available through wholesalers or the Colgate-Palmolive Co.

▶ Effective in the treatment of diarrhea are **Lactinex** ***Granules*** and ***Tablets***—products of **Hynson, Westcott & Dunning.**

▶ **Rhulicream** announced by **Lederle Laboratories** is an analgesic-anesthetic cream recommended for the relief of pain and itching from poison ivy, poison oak, insect bites, and other minor skin irritations.

▶ "Choice among liquid vitamins," states **Lilly,** "is *potent* **'Homicebrin,'** which provides *six* essential vitamins (including B_{12})."

▶ Counselling the maintenance of adequate stocks, the manufacturer catalogues the following **Massengill** prescription specialties: **Adrenosem, Aminodrox, Gallogen, Livitamin, Massengill Powder, Obedrin, Salcedrex** and **Semhyten.**

▶ **Geriplex** (vitamin-mineral formula) Kapseals are described by **Parke, Davis** as "a specialized R_x for the growing geriatric market." Each Kapseal contains eight vitamins, essential minerals, rutin and Taka-Diastase.

▶ **Pfizer Laboratories** labels **Toclase** as *new cough control.* It is available in three dosage forms—syrup, expectorant compound, and tablets. Pfizer's antibiotics, **Terramycin, Tetracyn,** and **Pen** are each tagged "SF"—Stress Fortified.

▶ **Smith, Kline & French Laboratories** re-emphasizes the ready saleability of its **Benzedrex Inhaler.**

▶ New sizes and forms of **Peritrate,** its prophylaxis against angina pectoris, and Methium, its hypotensive agent, are reflected in the **Warner-Chilcott** announcement.

▶ Reported to liquefy pulmonary secretions in asthma, bronchitis, croup, and laryngitis is **Alevaire,** product of **Winthrop-Stearns.**

Look for details in the Practical Pharmacy Edition of This Journal—Out April 20

Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

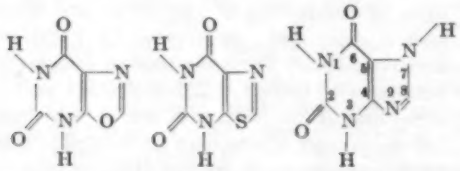
VOLUME XLIV — *APRIL, 1955* — NUMBER 4 — CONSECUTIVE NO. 8

Oxazolopyrimidine and Thiazolopyrimidine Derivatives Related to the Xanthines*

By GEORGE P. HAGER and CARL KAISER†

2,6(1H,3H)-Oxazolo[5.4-d]pyrimidinedione and 2,6(1H,3H)-thiazolo[5.4-d]-pyrimidinedione are xanthine analogs related to oxazolo[5.4-d]pyrimidine and thiazolo[5.4-d]pyrimidine in the same way that xanthine, 2,6(1H, 3H)-7-imidazo-[4.5-d]pyrimidinedione, is related to purine, 7-imidazo[4.5-d]pyrimidine. (The numbering employed for xanthine derivatives has been retained in naming the analogs.) In the analogs, the 7-imidazo ring of xanthine is replaced by an oxazole or thiazole ring. By substitution of hydrogens with methyl groups at positions 1 and 3 (as in theophylline or caffeine), or at position 1 only (as in theobromine), compounds analogous to the xanthines have been prepared. Related compounds substituted at position 8, are also described.

THE COMPOUNDS 2,6(1H, 3H)-Oxazolo[5.4-d]-pyrimidinedione (I)[1] and 2,6(1H,3H)-thiazolo-[5.4-d]pyrimidinedione (II) are analogous to xanthine, 2,6(1H,3H)-7-imidazo-[4.5-d]-pyrimidinedione (III) in that the 7-imidazo ring of the xanthine molecule is replaced by an oxazole or thiazole ring.

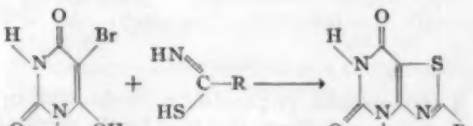


(I) (II) (III)

The analogs are related to oxazolo[5.4-d]pyrimidine (1) and thiazolo[5.4-d]pyrimidine (2) in the same way that xanthine is related to purine (7-imidazo[4.5-d]pyrimidine) (3).

By an appropriate distribution of methyl groups, compounds analogous to theophylline or caffeine as regards N-substituents in the pyrimidine ring (methyl groups at positions 1 and 3) and theobromine (a methyl group at position 1), would result. The objective of this investigation was the preparation of such theophylline and theobromine analogs. In the methods of synthesis so far employed, products bearing a methyl substituent at position 8 as well as those at positions 1 and 3 were more readily obtained, although several compounds with position 8 unsubstituted were prepared.

The condensation of 5-bromobarbituric acid with thioamides as indicated in Eq. 1, was reported to produce thiazolo[4.5-d]pyrimidinediones (4, 5):



(Equation 1)

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* Received August 27, 1954, from the School of Pharmacy, University of Maryland, Baltimore.

† H. A. B. Dunning Fellow, 1953–.

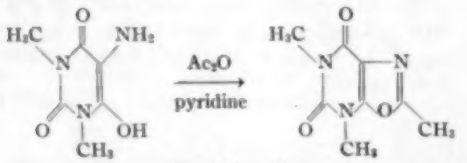
[1] The numbering employed for xanthine derivatives has been retained in naming the analogs.

Presented to the Scientific Section, A. PH. A., Boston meeting, August, 1954.

The condensation of 5-bromobarbituric acid with thioformamide yielded a product melting above 300°, as reported (4), but the product failed to give a qualitative test for sulfur. Similar results by other workers have recently been reported (6, 7). The attempted condensation of 5-bromobarbituric acid with thioacetamide resulted in the formation of barbituric acid and free sulfur as was also reported recently (8). Attempts to prepare 1-methyl- and 1,3-dimethyl-5-bromobarbituric acids by a previously reported procedure (9) were unsuccessful and these intermediates appear to be so much less stable than unsubstituted 5-bromobarbituric acid as to be useless in a reaction of the above type even if they had been obtained. The use of ammonium salts instead of the free acids in condensations with thioamides was unsuccessful. The unsubstituted 5-bromobarbituric acid was employed in a condensation with formamide and yielded a product having a nitrogen content corresponding to that calculated for 2,6(1H,3H)-oxazolo[4.5-d]pyrimidinedione monohydrate. Further study will be necessary to establish the identity of this compound.

Uramils and thiouramils may be used for the preparation of oxazolo- and thiazolo[5.4-d]-pyrimidinediones. 1,3-Dimethyluramil was prepared by oxidation of caffeine to 1,3-dimethylalloxan, reduction to tetramethylalloxantin, further reduction to 1,3-dimethyldialuric acid, and ammonolysis to 1,3-dimethyluramil essentially by a procedure described by Biltz and Damm (10). In the final step, ammonolysis of 1,3-dimethyldialuric acid by use of a solution of ammonia "acidified" with acetic acid, it was found to be necessary to adjust the *p*H at 7.5–7.8 by titration, otherwise the change in color of the reaction mixture from violet to yellow did not occur and no product was isolated.

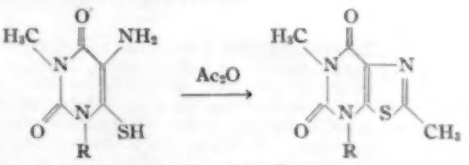
The 1,3-dimethyluramil was condensed with acetic anhydride in pyridine by a known method (11) producing 1,3,8-trimethyl-2,6-oxazolo[5.4-d]-pyrimidinedione, an 8-substituted analog of theophylline (Eq. 2).



(Equation 2)

1-Methyluramil, prepared from theobromine by a procedure similar to that used for the preparation of 1,3-dimethyluramil (12), was condensed with acetic anhydride to produce 1,8-dimethyl-2,6(3H)-oxazolo[5.4-d]pyrimidinedione, an analog of theobromine.

The preparation of 6-thiouramil and 1,3-dimethyl-6-thiouramil by treatment of the corresponding uric acid with ammonium sulfide solution at 135–140° in an autoclave has been described (13). This procedure was employed for the preparation of 6-thiouramil, 1-methyl-6-thiouramil, and 1,3-dimethyl-6-thiouramil. The procedure was modified by doubling the initial proportion of the appropriate uric acid in the reaction mixture because of the limited capacity of the reaction vessel, without decreasing the yield of the product. Higher proportions of the uric acids resulted in decreased yields. 1-Methyl- and 1,3-dimethyl-6-thiouramils were successfully condensed with acetic anhydride to produce 1,8-dimethyl-2,6(3H)-thiazolo [5.4-d]-pyrimidinedione (an analog of theobromine) and 1,3,8-trimethyl-2,6-thiazolo[5.4-d]pyrimidinedione (an analog of theophylline and caffeine) in accordance with Eq. 3.

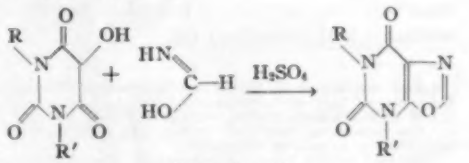


(Equation 3)

where R = H or CH_3

1,3-Dimethyl-2,6-thiazolo[5.4-d]pyrimidinedione, an analog of theophylline or caffeine unsubstituted in the 8-position, was obtained by a similar condensation of 1,3-dimethyl-6-thiouramil with formic acid. The condensation of 6-thiouramil with acetic anhydride produced a sample of 8-methyl-2,6(1H,3H)-thiazolo[5.4-d]pyrimidinedione which had all the physical characteristics of compounds of this series and which melted above 300°, as reported by Falco and Hitchings (14). The same product had earlier been reported to melt at 220–221° (13).

Because of the successful use of α-hydroxy ketones in place of α-halogen-substituted ketones for the preparation of oxazoles (15), the preparation of oxazolopyrimidinediones from the readily available and stable dialuric acids is being investigated (Eq. 4).



(Equation 4)

EXPERIMENTAL

All melting points were determined using a Fisher-Johns melting point apparatus, and they are uncorrected.

1,3-Dimethyluramil.—Prepared by a modification of a previously described procedure (10). 1,3-Dimethyldialuric acid was prepared from caffeine by oxidation to 1,3-dimethylalloxan, followed by reduction to tetramethylalloxantin (16), and further reduction to 1,3-dimethyldialuric acid (10). A mixture of 5 Gm. (0.029 mole) of 1,3-dimethyldialuric acid and 40 ml. of water was heated to boiling and 20 ml. of an ammonium acetate solution, which was prepared by titration of 20% ammonia solution to *p*H 7.5–7.8 with glacial acetic acid, was added. Boiling was continued until a considerable amount of crystals had formed. The mixture was then cooled and the product filtered and washed with a small amount of ethanol and ether. The yield was 2 Gm. (40%) of a product having physical properties identical with those described previously (10).

6-Thiouramil.—This compound was prepared by a modification of the procedure described by Fischer and Ach (13). Uric acid (10 Gm., 0.06 mole) was mixed with 100 ml. of cold water and 2.5 ml. of a 50% solution of potassium hydroxide was added, producing a thick paste of the potassium salt of uric acid. To this paste was added 160 ml. of a freshly prepared concentrated solution of ammonium sulfide (28% ammonia solution saturated with hydrogen sulfide while cooling). The mixture was thoroughly stirred and then heated in a closed reaction vessel for six hours at 160°. The reaction mixture was allowed to cool and the golden-yellow crystals of the ammonium salt of 6-thiouramil were removed by filtration. The yield was 6.65 Gm. (64%). The salt was converted to free 6-thiouramil by treatment with acetic acid. The product was identical in all respects with that previously described by Fischer and Ach (13). When the proportion of uric acid used in this procedure was doubled, the yield dropped to 55%; when it was tripled, the yield was 43%.

1-Methyl-6-thiouramil.—1-Methyluric acid (6.7 Gm.; 0.037 mole) was mixed with 40 ml. of a 2% ammonia solution and to the mixture was added 100 ml. of a freshly prepared concentrated solution of ammonium sulfide. The mixture was stirred thoroughly and then heated in a closed reaction vessel for six hours at 150°. The clear yellow solution was evaporated to dryness on a steam bath and the residue was recrystallized from approximately 250 ml. of water. The yield was 2.25 Gm. (38%) of small golden yellow crystals which did not melt up to 300°, but showed signs of gradual decomposition beginning at about 280°.

Anal.—Calcd. for $C_5H_7N_3O_2S$: N, 24.26. Found: N, 24.17.

1,3-Dimethyl-6-thiouramil.—Prepared by a modification of a previously described procedure (13). A mixture of 20 Gm. (0.1 mole) of 1,3-dimethyluric acid, 85 ml. of a 2% ammonia solution, and 160 ml. of a freshly prepared concentrated solution of ammonium sulfide was heated in a closed reaction vessel for two hours at 150°. The resulting clear solution was evaporated to dryness on a steam bath and the grayish-green residue (17.8 Gm.) was recrystallized from 1,300 ml. of water. The yield was approximately 15 Gm. (78%), light tan needles, m. p. 280–282° dec., reported m. p. *ca.* 300° with gradual decomposition beginning above 200° (13). The other physical properties of the product were the same as previously described (13).

2,6(1H,3H) - Oxazolo[4.5 - d]pyrimidinedione.—The 5-bromobarbituric acid used in this preparation was prepared by a previously described method (17). 5-Bromobarbituric acid (5 Gm.; 0.024 mole) was mixed with 2.25 Gm. (0.05 mole) of formamide and the mixture was heated on a boiling water bath for six hours. The solid residue was suspended in 50 ml. of boiling water, filtered, and washed with ethanol and ether. The yield was approximately 3 Gm. (83%) of a light tan powder which did not melt when heated to 300°. For analysis the above purification procedure was repeated twice.

Anal.—Calcd. for $C_5H_3N_3O_3.H_2O$: N, 24.50. Found: N, 24.20.[2]

1,8-Dimethyl-2,6(3H)-oxazolo[5.4-d]pyrimidinedione.—A mixture of 4 Gm. (0.026 mole) of 1-methyluramil, 50 ml. of acetic anhydride, and 4 ml. of pyridine was heated under reflux for five hours. The reddish-brown solution was concentrated as completely as possible by heating on a water bath under reduced pressure. The residue was taken up in approximately 15 ml. of dry acetone, and the product was precipitated as a dark oil by addition of isopropyl ether. By scratching the walls of the flask and chilling overnight in a refrigerator, a crystalline product was obtained. The product was immediately placed in a vacuum desiccator and dried. For analysis, the above purification procedure was repeated, producing light tan crystals, m. p. 133–135°. The yield was approximately 0.75 Gm. (16%).

Anal.—Calcd. for $C_7H_7N_3O_3$: N, 23.19. Found: N, 22.89.

8-Methyl-2,6(1H, 3H)-thiazolo[5.4-d]pyrimidinedione.—The ammonium salt of 6-thiouramil (5 Gm.; 0.028 mole) was heated under reflux with 150 ml. of acetic anhydride for thirty minutes. The crystalline product was filtered and recrystallized from water. The yield was 5 Gm. (96%) of fine white needles, m. p. above 300°; reported m. p. 220–221° (13) and above 340° (14). Other properties of the product such as solubility in water, solubility in alkali, oxidation by chlorine water to produce a product giving a murexide test, and formation of a silver salt, were the same as previously described (13).

1,8-Dimethyl-2,6(3H)-thiazolo[5.4-d]pyrimidinedione.—1-Methyl-6-thiouramil (1 Gm.; 0.006 mole) was heated under reflux with 10 ml. of acetic anhydride for four hours. The reaction mixture was filtered and the filtrate was chilled to produce tan needles. The product was recrystallized from ethanol or water producing fine, light tan needles, m. p. 188–189°. The yield was 1 Gm. (88%).

Anal.—Calcd. for $C_7H_7N_3O_2S$: N, 21.31. Found: N, 21.33.

1,3,8 - Trimethyl - 2,6 - thiazolo[5.4 - d]pyrimidinedione.—1,3-Dimethyl-6-thiouramil (2 Gm.; 0.011

[2] Further study, including an ultraviolet absorption spectra, will be necessary to establish the identity of this compound.

mole) and 15 ml. of acetic anhydride was heated under reflux for about thirty minutes. The solution was cooled and the crystalline precipitate removed by filtration. The product was recrystallized from ethanol producing 1.25 Gm. (55%) of fine white needles. After a second recrystallization from ethanol, the product melted at 197–198°.

Anal.—Calcd. for $C_8H_9N_3O_2S$: N, 19.89. Found: N, 20.03.

1,3-Dimethyl-2,6-thiazolo[5.4-d]pyrimidinedione.—1,3-Dimethyl-6-thiouramil (2 Gm.; 0.011 mole) was heated under reflux with 15 ml. of 90% formic acid for one and one-half hours. The reaction mixture was chilled and treated with an equal volume of water. The precipitated product was filtered and recrystallized from approximately 50 ml. of glacial acetic acid using a small amount of decolorizing carbon. The yield was approximately 0.5 Gm. (24%), small, light yellow prisms, m. p. 286.0–286.5°.

Anal.—Calcd. for $C_7H_7N_3O_2S$: N, 21.31. Found: N, 21.36.

SUMMARY

1. Series of oxazolopyrimidinediones and of thiazolopyrimidinediones, having N-methyl substituents so distributed that the substances are analogous to the xanthines (7-imidazopyrimidinediones), have been prepared.

2. Members of these series bearing a methyl substituent in position 8 were somewhat more readily obtained than the unsubstituted compounds.

3. The preparation of 1-methyl-6-thiouramil was accomplished using a modification of a procedure previously reported for related compounds.

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The Pharmacological Action of Chelerythrine*

By C. E. POWELL and K. K. CHEN

Chelerythrine hydrochloride, has been subjected to a pharmacologic study. Its action was determined on the blood pressure, uterus, and intestine, and the nictitating membrane of eighteen cats and one dog. Chelerythrine lowered the blood pressure in anesthetized cats, preceded by a brief pressor action. Initially it blocked respiration, which was followed by an increase in rate and amplitude. The alkaloid initially stimulated the isolated small intestine and isolated uterus of rabbits and guinea pigs, but in repeated doses it became less effective. Administered by vein, chelerythrine caused the Harderian gland of guinea pigs to extrude a creamy white secretion and in rats and hamsters a clear watery excretion.

A CONSIDERABLE NUMBER OF PLANTS belonging to the *Papaveraceae* family are known to contain alkaloids. In this family, the genus *Bocconia*, which includes nine species, is found in Mexico and in South American countries. Murrill and Schlotterbeck (1), isolated and identified four separate alkaloids from *B. cordata*. They were named protopine, β-homochelidonine, chelerythrine, and sanguinarine. From the leaves of *B. frutescens*, Miller (2) isolated four separate alkaloids, one of which was identified as chelerythrine. Manske (3) obtained an 0.86 per cent yield of chelerythrine from *B. arborea;* and Maccio (4) a 1 per cent chelerythrine from *B. pearei*. Chemically, Henry (5) described chelerythrine as a quaternary base belonging to the α-naphtha-phenanthridine (1:2-benzphenanthridine) group. Attempts of synthesis have been undertaken by Richardson, *et al.* (6), and by Bailey and Robinson (7, 8).

Dragendorff (9) reported the juice of leaves and the bark of *B. arborea* to have a laxative and anthelmintic action. A more extensive study by

* Received November 5, 1954, from the Lilly Research Laboratories, Indianapolis 6, Ind.
We wish to acknowledge the valuable assistance of Messrs. Lester LeCompte, Jr., and James E. Waddell.

Madaus (10) showed that chelerythrine had an irritating and inflammatory effect on the skin and mucous membranes because it caused blistering. The paucity of pharmacological information prompted us to undertake the present investigation.

EXPERIMENTAL

Material.—Our sample of chelerythrine in the form of hydrochloride, isolated from a Mexican species of *Bocconia*, was generously provided by Dr. Carl Djerassi, Wayne University, Detroit. The alkaloid had an orange color, melted at 203–204°, and was easily soluble to 1% in distilled water.

The pharmacologic action of chelerythrine was determined on the blood pressure, uterus, and intestine, and the nictitating membrane of 18 cats and one dog. The animals were anesthetized with either Dial®-urethane or with sodium phenobarbital. In all experiments doses ranging from 1 to 10 mg. per Kg. of the hydrochloride were given through the femoral vein, and blood pressure was recorded from the cannulated carotid artery. The action of chelerythrine on the uterus, intestine, and nictitating membrane was registered through a pulley system. Electrocardiographic studies on two cats and one dog were performed with 2, 5, and 10 mg. per Kg. intravenous doses of chelerythrine, respectively.

Eight uterine and 14 small intestinal strips were isolated from nine guinea pigs; 11 uterine and 16 intestinal strips, from 10 rabbits; and nine uterine strips, from six rats. The isolated organ in each case was suspended in a 100-cc. muscle chamber and movements recorded on a smoked paper kymograph by a gravity lever. The muscles were bathed in Tyrode's solution maintained at a temperature of 37.5°. Dilutions of chelerythrine ranging from 1:10,000 to 1:400,000 were used.

Toxicity was determined by injecting the alkaloid into the tail vein of starved albino mice weighing 15 to 29 Gm., and the saphenous vein of guinea pigs weighing 270–295 Gm. The median lethal dose was calculated by the method of Miller and Tainter (11).

The action of the alkaloid was also tested on 13 hamsters which were given doses ranging from 10 to 30 mg. per Kg. by the saphenous vein.

RESULTS AND DISCUSSION

Action in Anesthetized Animals.—During and following injection of each dose of chelerythrine, there was a brief stoppage of respiration followed by a slight increase in rate and amplitude. Doses of 2.5 to 10 mg. per Kg, by vein consistently caused a sustained lowering of blood pressure (14–50 mm. Hg) preceded by a brief pressor response with an increase in heart rate. The duration of action varied from ten to thirty minutes.

Doses of 1 mg./Kg. and 2 mg./Kg. elicited inconsistent responses of the blood pressure. Out of a total of 12 doses, seven resulted in a depressor response, four in a pressor response, and one in a pressor followed by a depressor response. A 2-mg. dose of atropine by vein did not alter the blood-pressure-lowering action of 2.5 mg. per Kg. of chelerythrine. In the doses used, chelerythrine did not alter the effects of epinephrine and histamine on the blood pressure. Figure 1 shows the action of chelerythrine in an anesthetized cat.

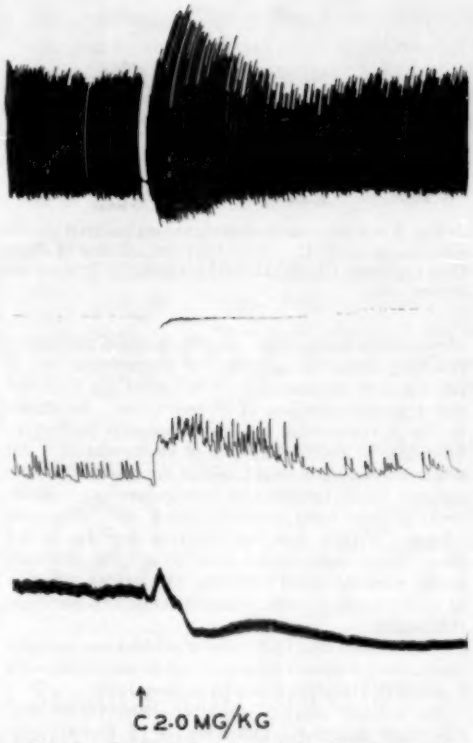

Fig. 1.—Action of chelerythrine in an anesthetized cat. Cat, male, 3.5 Kg., Dial anesthesia, 1 cc. per Kg. intraperitoneally. Tracings from top to bottom are: respiration, nictitating membrane, small intestines, blood pressure, base line and time in minutes. Note, chelerythrine (*C*) causes a sustained fall of blood pressure after a brief rise. Initially the respiration is blocked, followed by an increased rate and amplitude. The small intestines are stimulated while the nictitating membrane is relaxed.

Doses of 2 mg./Kg. to 4 mg./Kg. by vein had a slight stimulating action on the intact uterus of four cats—the response being principally that of increased rhythm.

In six cats, doses of 2 to 5 mg. per Kg. caused a slight relaxation of the nictitating membrane. No blocking of epinephrine- or histamine-induced stimulation was noted.

The motility of the intact intestine was increased following doses of 2 to 5 mg. per Kg. of chelerythrine by vein.

The electrocardiograms of one dog and two cats showed similar responses. Following doses of 2, 5, and 10 mg. per Kg. injected intravenously, there was in each case an increase in voltage of *P* and *T* waves. In the cats, there was a flattening, and at times an inversion of the *T* wave as well as inversion of the QRS complex. There were no arrhythmias.

Action on Isolated Smooth-Muscle Organs.—Dilutions of 1:100,000–1:10,000 of chelerythrine caused only a slight contraction of the isolated

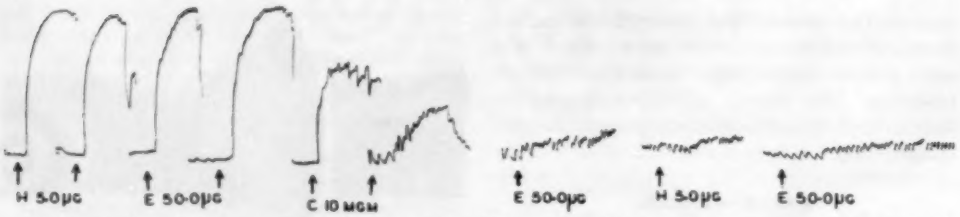

Fig. 2.—Action of chelerythrine on isolated guinea pig's uterus. A strip of uterus suspended in Tyrode's solution at 37.5° C. Note that the efficacy of chelerythrine (*C*) decreases with repeated doses. Note also that exposure to the alkaloid completely desensitizes the uterus to the effect of histamine (*H*) and of ergonovine (*E*).

uterus of the guinea pig. Contractions of the uterus resulting from 50 μg. doses of ergonovine and 5 μg. doses of histamine were inhibited by 1:10,000 and 1:20,000 dilutions of chelerythrine. As shown in Fig. 2, ergonovine and histamine were ineffective following 1:10,000 dilutions of chelerythrine. All dilutions caused a slight initial contraction of the isolated small intestine of the guinea pig. However, in some cases, repeated doses were either not effective or they were less effective than the initial dose. Also, contractions from 10 μg. doses of histamine were inhibited following a 1:200,000 dilution of chelerythrine. The alkaloid tended to paralyze this organ.

The isolated small intestine of rabbits was initially contracted, followed by a decrease in amplitude with 1:400,000–1:50,000 doses of chelerythrine.

On isolated uteri of rabbits, chelerythrine was relatively inactive. Dilutions of 1:10,000 only slightly contracted the muscle. Doses of 1:400,000 to 1:33,000 caused delayed inhibition of normal rhythmic movements of rats' uteri.

Toxicity.—The median lethal dose (LD_{50}) of chelerythrine in starved albino mice was determined to be 23.95 ± 1.75 mg. per Kg. during a twenty-four hour observation period. Immediately following the injection of lethal and sublethal doses the animals reacted with severe tonic convulsions, an increased rate of respiration, protrusion, and darkening of eyeballs. Surviving animals were depressed for a period of twenty-four hours. Animals that died during the twenty-four hour post-injection period were subjected to necropsy. Four mice, which received doses of 25, 27.5, 30, and 40 mg. per Kg., were found to have pulmonary edema. One surviving mouse, which had received a 25-mg. dose, was sacrificed. Histological examination showed necrosis of renal tubules, hydrops of liver, and slight fatty metamorphosis of the liver. In another surviving mouse, which had received 16 mg./Kg., only slight fatty metamorphosis of the liver was found. Post-mortem examination of four mice, which survived 16, 20, 25, and 27.5 mg. per Kg., revealed no abnormalities. The lesions, when detected, may not have been attributable to chelerythrine because they were inconsistent and had no relation to the size of the dose.

In guinea pigs, the median lethal dose (LD_{50}) of chelerythrine was determined to be 7.96 ± 0.65 mg. per Kg. Within one minute of the intravenous injection of 5 to 15 mg. per Kg. doses, an extrusion of a creamy secretion appeared in both eyes of the guinea pigs and lasted from one to two minutes. The animals became excitable; rubbed their noses with the forepaws; respiration increased, with gasping followed by general depression. In most cases urination occurred immediately after injections. Figure 3 shows the condition of the eye of a guinea pig after administration of 10 mg./Kg. dose of chelerythrine.

Fig. 3.—Photograph of guinea pig's eye. Guinea pig, male, weight 285 Gm. was injected intravenously with 10 mg./Kg. of chelerythrine. Note the extrusion of a creamy substance from the eye.

Histological examination showed that the secretion originated from the Harderian gland. Anatomically the gland was observed to be a chalky-white, kidney-shaped, and multilobular organ, occupying approximately 7/8 of the medial portion of the orbital floor. Glandular secretions flow through a duct opening on the nictitating membrane at the medial canthus. The entire apparatus has been described by Lowenthal (12).

When chelerythrine was given by vein to four rats in doses of 20 mg./Kg. and 30 mg./Kg., the animals reacted with general depression, increased respiration and the appearance of clear tears as opposed to the creamy secretion observed in guinea pigs. Death of the rats occurred within three hours. Smaller doses, 10, 12.5, and 16 mg. per Kg. by vein produced no noticeable effect on the animals.

Immediately following the injection of chelerythrine to the hamsters, the animals' eyes became watery and their noses turned a bright red and appeared to be swollen. Respiration was increased followed by a general depression of the animal. Doses of 20 mg./Kg. and 30 mg./Kg. were lethal. Chelerythrine is not the first substance to cause an

extrusion of Harderian secretion. Tashiro and Stix (13) reported that choline poisoning stimulated the flow of brick-red tears in rats. Towbin, *et al.* (14), concluded that the deep red tears in rats poisoned with choline contained a pigment identified as protoporphyrin. This pigment, and not hemoglobin, is responsible for the red color.

SUMMARY

Chelerythrine, an alkaloid of a Mexican species of *Bocconia*, has been shown to lower the blood pressure in anesthetized cats, preceded by a brief pressor action. Initially it blocks respiration, followed by an increase in rate and amplitude. Chelerythrine in the form of hydrochloride initially stimulates the isolated small intestine and isolated uterus of rabbits and guinea pigs, but repeated doses are less effective and block or partially inhibit the stimulating action of ergonovine maleate, epinephrine, and histamine acid phosphate—possibly due to the paralysis of smooth muscles. The normal rhythmic movements of the rat uterus are reduced by chelerythrine. Chelerythrine hydrochloride by vein causes the Harderian gland of guinea pigs to extrude a creamy white secretion; a clear watery excretion is expelled from the rat's and hamster's Harderian gland when similarly injected with the same alkaloid.

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Further Toxicological Studies with Ilotycin® (Erythromycin, Lilly)*

By ROBERT C. ANDERSON, PAUL N. HARRIS, and K. K. CHEN

Previously, we have shown that Ilotycin® (Erythromycin, Lilly), administered orally for three months, was well tolerated by laboratory animals. We now report similar results after daily administration for one year. In contrast to certain other antibiotics, Ilotycin® as the glucoheptonate salt produced no toxic manifestations following daily intravenous injections for three months in dogs. Also, Ilotycin® has been administered intramuscularly in dogs daily for four months with no apparent visceral damage.

ERYTHROMYCIN was discovered by members of the Lilly Research Laboratories and its properties were first disclosed by McGuire, *et al.* (1). It has had widespread clinical use, and the toxic manifestations observed have been few and mild. These consisted of very slight diarrhea in a relatively small number of patients. We reported on the low order of toxicity in laboratory animals when chronic toxicity tests were of three months' duration (2). The results of studies extending over one year are presented in this paper.

When Ilotycin® (Erythromycin, Lilly) was introduced for clinical use two years ago, only an acid-resistant coated tablet was available. Since then, the Lilly Pharmaceutical Research Department has developed regular and ophthalmic ointments, ear drops, Ilotycin® Glucoheptonate (Erythromycin Glucoheptonate, Lilly) for intravenous use, Ilotycin® Ethyl Carbonate (Erythromycin Ethyl Carbonate, Lilly) for pediatric use, and Ilotycin® (Erythromycin, Lilly). Intramuscular for parenteral administration. Toxicity studies carried out during the development of these forms will be included.

EXPERIMENTAL

Chronic Toxicity Studies by Oral Administration.—We have reported earlier (2) that rats fed diets containing as much as 0.2% Ilotycin® for three

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TABLE I.—BODY WEIGHT OF DOGS AFTER ORAL CHRONIC TOXICITY STUDIES FOR ONE YEAR

Dog No.	Daily Doses, mg./Kg. 3 Mo.	9 Mo.	Body Weight, Kg. Original	3 Mo.	12 Mo.
209	50	100	7.6	12.2	12.1
509	50	100	8.2	8.1	8.4
498	75	100	8.5	10.5	10.8
535	75	100	4.2	5.1	6.5
481	100	100	8.5	9.2	9.6
484	100	100	5.0	5.8	6.0
495	Control		7.6	9 6	11.1

TABLE IV.—ACUTE TOXICITY OF ILOTYCIN® GLUCOHEPTONATE IN LABORATORY ANIMALS FOLLOWING INTRAVENOUS INJECTION

Species	$LD_{50} \pm$ S.E., mg./Kg.
Mouse	453.0 ± 13.2
Rat	287.6 ± 6.0
Rabbit	>300

compiled in Table II, and nonprotein nitrogen and blood sugar levels, whole blood clotting and clot

TABLE II.—HEMATOPOIETIC STUDIES ON DOGS, CONTROL, AND AFTER ONE YEAR ON ILOTYCIN®

Dog No.	Date	Hbg., Gm./100 cc.	R.B.C. (Million)	W.B.C. (Thousand)	Lymphs., %	Polys., %	Eosino., %	Mono., %	Blast., %
209	3-31-52[a]	14.2	6.24	17.1	7	80	4	9	..
	3-31-53	17.2	10.64	8.4	17	75	7	1	..
509	3-31-52[a]	14.0	6.51	18.3	4	60	16	18	2
	3-31-53	16.0	7.72	11.8	17	63	17	2	..
498	3-31-52[a]	15.4	6.55	23.3	12	66	18	3	1
	3-31-53	13.8	7.94	19.3	13	69	14	4	..
535	3-31-52[a]	13.4	6.40	15.2	3	84	5	6	2
	3-31-53	14.2	7.06	11.9	17	68	12	1	2
481	3-31-52[a]	14.6	6.47	25.4	8	78	12	2	..
	3-31-53	14.9	6.47	15.4	26	60	14	2	..
484	3-31-52[a]	14.5	6.12	22.7	8	77	11	3	1
	3-31-53	13.9	5.97	16.3	14	50	32	1	2
495	3-31-52[a]	12.2	6.02	17.6	6	83	3	5	3
	3-31-53	12.2	6.38	17.4	16	68	12	2	2

[a] Control.

TABLE III.—BLOOD AND URINE ANALYSES ON DOGS, CONTROL, AND AFTER ONE YEAR ON ILOTYCIN®

Dog No.	Date	Blood Sugar, mg./100 cc.	NPN, mg./100 cc.	Whole Blood Clotting Time, min.	Clot Retraction Time, hr.	Urine Sugar	Urine Albumin
209	3-31-52	95	47.0	..	..	+	−
	3-31-53	90	40.0	23	4	−	−
509	3-31-52	79	25.2	..	..	−	+
	3-31-53	70	38.0	21	2	−	+
498	3-31-52	84	33.6	..	..	−	+
	3-31-53	70	40.8	14	3	−	+
535	3-31-52	93	30.8	..	..	−	−
	3-31-53	95	41.3	23	4	−	−
481	3-31-52	86	33.3	..	..	+	−
	3-31-53	86	33.3	28	4	−	−
484	3-31-52	86	32.5	..	..	−	−
	3-31-53	93	38.0	16	4	−	−
495	3-31-52	90	29.7	..	..	+	−
	3-31-53	90	33.0	21	4	−	−

months were normal by all criteria, i. e., the growth curve was identical with that of a control group, hematopoietic studies were normal, and no visceral damage was found on histological examination. Also, 9 of the 15 dogs, treated orally twice daily at three dose levels and sacrificed after ninety-one days and submitted for necropsy, presented neither hematopoietic nor visceral damage.

Although there was no indication of toxic effects, the remaining six dogs were treated for an additional nine months with doses of 50 mg./Kg. twice daily. One untreated dog was studied for the same period as a control. The effects on the body weights are found in Table I. Blood counts, blood, and urine analyses were continued semiweekly. Erythrocyte, leukocyte, and differential counts, and hemoglobin values prior to and after one year of medication are retraction times, and urine sugar and albumin estimates, in Table III.

One year after the start of medication, the six treated dogs and the untreated control were sacrificed and submitted for necropsy. Gross and microscopic studies of the heart, lungs, liver, spleen, kid-

TABLE V.—BODY WEIGHT OF DOGS AFTER DAILY INTRAVENOUS INJECTIONS OF ILOTYCIN® GLUCOHEPTONATE FOR THREE MONTHS

Dog No.	Daily Dose, mg./Kg.	Body Weight— Initial, Kg.	Necropsy, Kg.	No. of Doses Tolerated	Results
3890	170	9.6	10.3	92	Survived
3902	170	9.9	10.7	92	Survived
3904	170	11.1	11.7	92	Survived

TABLE VI.—HEMATOPOIETIC STUDIES ON DOGS, CONTROL, AND AFTER THREE MONTHS OF DAILY INTRAVENOUS INJECTIONS

Dog No.	Date	Hemat., %	Hbg., Gm./100 cc.	R.B.C. (Million)	W.B.C. (Thousand)	Lymphs., %	Polys., %	Eosino., %	Mono., %	Myelo., %	Blast., %
3890	1-6-54	45	12.9	6.19	19.2	6	90	2	1	1	..
	4-8-54	45	13.1	5.73	16.4	25	61	3	..	11	..
3902	1-6-54	45	12.1	6.62	15.9	10	80	6	1	1	2
	4-8-54	44	13.0	6.88	14.3	13	69	10	..	7	1
3904	1-6-54	46	13.7	6.95	15.8	13	79	6	..	2	..
	4-8-54	47	13.7	6.72	18.2	44	36	12	..	8	..

TABLE VII.—BLOOD AND URINE ANALYSES ON DOGS, CONTROL, AND AFTER THREE MONTHS OF DAILY INTRAVENOUS INJECTIONS

Dog No.	Date	Blood Sugar mg./100 cc.	NPN mg./100 cc.	Whole Blood Clotting Time, min.	Clot Retraction Time, hr.	Urine Sugar	Urine Albumin
3890	1-6-54	66	42.8	16	1¼	−	++
	4-8-54	72	30.8	15	1¾	++	−
3902	1-6-54	75	39.4	12	1	−	++++
	4-8-54	88	33.3	13	2	−	−
3904	1-6-54	61	44.5	13	4	−	+−
	4-8-54	70	31.0	23	2½	−	−

neys, stomach, intestines, thymus, pancreas, thyroid, and adrenal glands revealed no abnormalities. Bone marrow studies were made at the same time. The myeloid, erythroid, and lymphoid cell counts were comparable to those found in untreated dogs.

From the results summarized above it is obvious that Ilotycin® administered to dogs orally in large divided doses daily for one year caused no hematopoietic or visceral damage.

Ilotycin® Glucoheptonate for Intravenous Injection.—In developing a salt for intravenous use, the glucoheptonate was found to have the necessary attributes, such as solubility, stability, and lack of irritating properties. The acute toxicities after intravenous injection in mice, rats, and rabbits are presented in Table IV. The values are comparable to those previously published for the hydrochloride (2).

(*a*) *Subacute Toxicity.*—Sedwitz, *et al.* published a report on the toxicity of oxytetracycline in dogs after intravenous administration (3). They reported that five dogs on daily doses of 25 to 75 mg. per Kg. of oxytetracycline all succumbed in four to seven days after showing symptoms of increasing anorexia, lethargy, and azotemia. Nonprotein nitrogen levels rose to 251 mg. per 100 cc., but renal damage was not found. It was postulated that death resulted from systemic toxicity when the rate of drug administration exceeded that of excretion and detoxification.

For comparative purposes we injected three female mongrel dogs by vein twice daily for three months with doses of 85 mg./Kg. of Ilotycin® Glucoheptonate, or a total of 170 mg./Kg. daily. During the early days of the study, the dogs had mild tremors and emesis soon after each injection. However, these reactions disappeared and no other side-effects were noted. The effect on body weights are found in Table V. Blood and urine studies, like those outlined previously, were made at weekly intervals. The control figures and those obtained at necropsy are compared in Tables VI and VII. Complete histological examination, both gross and microscopic, revealed no abnormalities. Terminal bone marrow counts were unaltered.

The results show that dogs that received as much as 77 Gm. of Ilotycin® Glucoheptonate by vein over a three-month period remained normal.

(*b*) *Excretion Studies.*—A product such as Ilotycin® Glucoheptonate is intended primarily for intravenous infusion, but may be used at times by direct injection. The serum levels and urinary excretions following each type of administration are compared in Table VIII. Ilotycin® Glucoheptonate was dissolved in water and diluted with physiological salt solution to a concentration of 0.03%. Dog No. 233 received a dose of 5 mg./Kg. in terms of the base. The intravenous drip was controlled so that about 2.5 cc. were administered per minute with the entire dose requiring ninety minutes. Dog No. 262 received a dose of 10 mg./Kg. in the same manner except that the rate of infusion was doubled. For direct injections, 2.5% aqueous solutions were used.

As expected, higher initial levels were obtained by direct injection; however, serum levels soon fell to comparable values.

It is interesting that some Ilotycin® was also found in the feces after intravenous injection. This apparently originated in the bile as studies from this laboratory have shown as much as 50% of the injected dose of Ilotycin® Glucoheptonate can be recovered in the bile and urine within six hours after injection (4).

Ilotycin® Intramuscular for Parenteral Use.—Aqueous preparations of many antibiotics are so irritating as to preclude parenteral administration. When diethyl carbonate was substituted as the vehicle, a satisfactory parenteral product of Ilotycin® could be compounded. The formulation is as follows:

Ilotycin® base	50 mg./cc.
Digammacaine	20 mg./cc.
Phenol	5 mg./cc.
Diethyl carbonate	*q. s.*

TABLE VIII.—SERUM AND URINE LEVELS IN DOGS AFTER ADMINISTRATION OF ILOTYCIN® GLUCOHEPTONATE INTRAVENOUSLY AND BY INFUSION

Time after Start of Administration	Intravenous Infusion: Serum Level, μg./ml.	Intravenous Infusion: Urine Level, μg./ml.	Intravenous Infusion: Urine Total Excreted, μg.	Intravenous Injection: Serum Level, μg./ml.	Intravenous Injection: Urine Level, μg./ml.	Intravenous Injection: Urine Total Excreted, μg.
Dog No. 233, 8.5 Kg., 5 Mg./Kg. (in Terms of Base)						
15 min.	0.64	..	..	5.12	..	..
30 min.	0.64	..	..	..	..	..
45 min.	1.28	..	..	..	..	..
60 min.	1.28	..	..	..	..	..
75 min.	1.28	..	..	2.56	98	4018
90 min.	1.28	62.5	2600	..	..	..
3 hr.	0.16	<25	..	0.32	79	1106
5 hr.	0.12	<25	..	0.08	..	..
7 hr.	..	..	..	0.04	..	..
22½ hr.	<0.06	<25	..	..	..	..
	Recovery urine >2.6 mg.					5.1 mg.
	Recovery feces 4.5 mg.					
Dog No. 262, 9.4 Kg., 10 Mg./Kg. (in Terms of Base)						
15 min.	2.56	..	..	5.12	..	..
30 min.	3.84	..	..	..	..	..
45 min.	3.84	..	..	..	..	..
60 min.	3.84	..	..	..	..	..
75 min.	1.92	..	..	2.56	675	8100
90 min.	1.92	64	7808	..	..	..
2½ hr.	0.96	<25	..	0.64	363	2178
5 hr.	..	..	..	0.32	256	1548
7 hr.	..	..	..	0.08	62	558
18½ hr.	<0.06	<25	..	..	..	..
	Recovery urine >7.8 mg.					12.4 mg.
	Recovery feces 7.5 mg.					

TABLE IX.—ACUTE TOXICITY OF DIETHYL CARBONATE ALONE, AND IN COMBINATION WITH DIGAMMACAINE AND WITH DIGAMMACAINE PLUS ILOTYCIN® FOLLOWING SUBCUTANEOUS INJECTION IN MICE

Preparation	LD_{50} ± S.E.[a]: Ilotycin®, mg./Kg.	Digammacaine, mg./Kg.	Diethyl Carbonate, cc./Kg.
Diethyl carbonate	.....	.....	9.61 ± 1.87
Digammacaine in diethyl carbonate	.....	162.2 ± 15.6	8.1
Ilotycin®, Intramuscular	393.8 ± 31.5	157.5	8.5

[a] This was the amount of each ingredient in one LD_{50} of the preparations listed on the left.

TABLE X.—EFFECT OF DAILY INTRAMUSCULAR INJECTIONS OF AMPUL ILOTYCIN® AND DIETHYL CARBONATE ON DOGS

Dog No.	Daily Dose: Ilotycin®, mg./Kg.	Daily Dose: Digammacaine mg./Kg.	Daily Dose: Diethyl Carbonate, cc./Kg.	No. of Doses Tolerated	Body Weight: Initial, Kg.	Body Weight: Current, Kg.	Results
3841	..	..	0.05	112[a]	6.5	7.4	On test
4104	..	..	0.05	112	6.7	10.3	Sacrificed
4460	..	..	0.05	112[a]	5.9	7.0	On test
4462	..	..	0.10	8[b]	6.7	5.4	Sacrificed
4481	..	..	0.10	112	8.0	10.0	Sacrificed
4514	..	..	0.10	112	8.2	9.7	Sacrificed
4211	5	2	0.10	112[a]	6.5	9.2	On test
4213	5	2	0.10	112[a]	6.3	6.6	On test
4214	5	2	0.10	112[a]	8.6	9.2	On test
4215	10	4	0.20	112	6.0	6.4	Sacrificed
4220	10	4	0.20	112	8.3	9.2	Sacrificed
4221	10	4	0.20	112	5.2	9.4	Sacrificed

[a] Dogs remain on test. [b] Developed pneumonia and was sacrificed.

(*a*) *Acute Toxicity.*—The LD_{50} of the combination and of its individual principal ingredients are found in Table IX. As is shown there, the addition of 2% Digammacaine[1] in one case or the addition of 2% Digammacaine and 5% Ilotycin® did not materially change the dose of diethyl carbonate that was toxic. The toxicity of the mixture is less than expected if the lethality of the constituents were additive.

(*b*) *Subacute Toxicity.*—These studies were similar to those previously described. Twelve female mongrel dogs were given daily intramuscular injec-

[1] Digammacaine is 1-benzamido-1-phenyl-3-piperidinopropane hydrochloride.

TABLE XI.—HEMATOPOIETIC STUDIES ON DOGS AFTER DAILY INTRAMUSCULAR INJECTIONS OF ILOTYCIN® INTRAMUSCULAR AND DIETHYL CARBONATE FOR FOUR MONTHS

Dog No.	Date	Hemat. %	Hbg., Gm./100 cc.	R.B.C. (Million)	W.B.C. (Thousand)	Lymphs., %	Polys., %	Eosino., %	Mono., %	Myelo., %	Blast., %
3841	3-17-54[a]	41	11.9	6.31	30.8	20	62	5	..	12	1
	7-22-54	52	16.2	7.60	19.6	23	60	14	..	3	..
4104	3-17-54[a]	44	11.2	5.56	19.7	19	67	6	..	8	..
	7- 8-54	48	14.1	8.19	15.7	20	75	1	..	4	..
4460	3-17-54[a]	44	13.0	7.23	21.0	21	63	..	1	11	5
	7-22-54	54	17.2	8.64	21.6	35	36	26	1	2	..
4481	3-17-54[a]	48	15.5	6.87	13.3	16	65	4	3	8	4
	7- 8-54	52	16.0	6.75	14.7	37	50	11	1	1	..
4514	3-17-54[a]	44	12.9	6.42	19.8	17	62	3	1	13	4
	7- 8-54	51	16.7	8.04	22.3	27	65	2	1	5	..
4211	3-10-54[a]	46	12.0	6.48	27.6	10	84	3	1	2	..
	7-22-54	51	16.5	6.76	15.5	43	40	12	1	4	..
4213	3-15-54[a]	50	14.0	6.30	14.6	15	74	3	..	7	1
	7-22-54	47	14.0	7.70	11.2	24	61	12	1	2	..
4214	3-15-54[a]	46	13.8	6.29	22.2	9	66	18	1	5	1
	7-22-54	42	13.0	5.71	26.1	23	47	23	..	7	..
4215	3-15-54[a]	46	12.7	5.90	25.0	17	70	8	..	5	..
	7- 8-54	47	14.1	6.85	26.3	31	48	18	..	2	1
4220	3-15-54[a]	55	15.7	7.56	18.7	30	61	3	..	6	..
	7- 8-54	44	13.4	6.56	12.8	33	60	4	..	3	..
4221	3-15-54[a]	42	..	5.92	13.3	12	80	5	..	3	..
	7- 8-54	47	14.4	6.19	10.8	25	64	9	..	1	1

[a] Control.

TABLE XII.—BLOOD AND URINE ANALYSES ON DOGS AFTER DAILY INTRAMUSCULAR INJECTIONS OF ILOTYCIN® INTRAMUSCULAR AND DIETHYL CARBONATE FOR FOUR MONTHS

Dog No.	Date	Blood Sugar, mg./100 cc.	NPN, mg./100 cc.	Whole Blood Clotting Time, min.	Clot Retraction Time, hr.	Urine Sugar	Urine Albumin
3841	3-17-54	72	33.6	15	2	−	−
	7-22-54	79	42.2	20	1½	++++	−
4104	3-17-54	72	29.9	18	2	+−	−
	7- 8-54	83	35.8	10	2	−	−
4460	3-17-54	68	23.2	20	2	+++	−
	7-22-54	74	31.9	19	1½	−	−
4481	3-17-54	75	31.6	18	2	+	+
	7- 8-54	79	42.0	20	3	−	−
4514	3-17-54	68	31.9	16	2½	++	−
	7- 8-54	93	47.6	14	2	−	−
4211	3-10-54[a]	83	38.6	16	2	−	−
	7-22-54[a]	86	33.6	13	1½	−	−
4213	3-15-54[a]	75	22.6	20	2	−	−
	7-22-54[a]	75	28.8	11	1½	++	−
4214	3-15-54[a]	70	35.0	21	2	−	−
	7-22-54[a]	86	37.2	12	1	+++	−
4215	3-15-54[a]	72	29.9	26	2	−	−
	7- 8-54[a]	79	33.8	9	1	+	++++
4220	3-15-54[a]	68	29.6	16	2	−	−
	7- 8-54[a]	88	35.0	12	1	−	++
4221	3-15-54[a]	61	33.0	14	2	+−	−
	7- 8-54[a]	81	35.2	23	1	+++	−

[a] Control.

tions for four months. Six received the Ilotycin® Intramuscular and six, the solvent alone. The results appear in Table X. Again, blood and urine samples were obtained semiweekly for analyses. The control levels and those found four months later are recorded in Tables XI and XII. Six dogs were sacrificed and submitted for histological studies. No damage to the parenchymatous organs was found. Bone marrow counts remained unaltered. The remaining six dogs are still on test.

SUMMARY

1. The acute and chronic toxicity of Ilotycin® has been determined by oral, intravenous, and intramuscular routes.

2. Ilotycin® is well tolerated by dogs by all three modes of administration. No damage to the viscera or the hematopoietic system has been found.

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The Polarographic Determination of Acriflavine*

By ARTHUR J. ZIMMER† and KHALID MANSUR‡

The polarographic behavior of acriflavine has been investigated. Acriflavine behaves polarographically as a single compound, and polarographic curves are suitable for analytical determinations. The effect of *p*H and concentrations of acriflavine on the curve is presented as well as results obtained from the use of the method for the quantitative determination of acriflavine in powder, tablet, and ointment forms. These results indicate that acriflavine can be determined successfully by the polarographic method.

IN THE PROCESS of the development of some acriflavine ointments, the need arose for the analysis of acriflavine in the presence of substances that interfere with the N. F. IX method (1). The determination of acriflavine in tablets by the gravimetric precipitation of the chloride as silver chloride allows for an accuracy of only plus or minus 20 per cent. The desirability of having a direct method for the determination of acriflavine is obvious.

The polarographic reduction of several acridine derivatives with reference to their antiseptic activity has been reported (2). Acriflavine was not included in the investigation. The authors report that the acridines are reduced in two stages, forming the mono- and dihydro-acridine radicals, respectively. The diffusion currents and concentrations were found to be mutually proportional, for most of the acridines studied, over a hundredfold range.

Even though acriflavine is a mixture of two substituted acridines, it was felt that polarographically it would act as a single compound, since the difference between the two acridines is that one has a methyl group at the 10 position and the other does not.

The purpose of this investigation is to study the polarographic behavior of acriflavine, particularly with regard to the utilization of this method for the determination of acriflavine in pharmaceutical preparations.

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Taken in part from a thesis presented in partial fulfillment of the requirements for the degree of Master of Science in Pharmacy to the St. Louis College of Pharmacy and Allied Sciences by Khalid Mansur.
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Presented to the Scientific Section, A. PH. A., Boston meeting, August, 1954.

EXPERIMENTAL

Apparatus and Materials.—A Sargent Model III manually operated polarograph was used. All potentials were measured against the saturated calomel electrode. The dropping mercury electrode had the following characteristics: drop time with the circuit open and the mercury at a height of 40 cm. was four seconds in water; the mass of mercury per second was 1.56 mg. The value of $m^{2/3}t^{1/6}$ was 1.70 $mg.^{2/3}$ $sec.^{1/6}$. Oxygen was removed by bubbling nitrogen through the solution for ten minutes or longer. The H cell designed by Lingane and Laitinen (3) was used. The temperature was maintained at 25° within 0.1° by immersing the electrolysis cell and electrode in a water bath kept at this temperature. The sensitivity of the polarograph was set at 5 for all determinations of the data presented here.

Buffer solutions were made according to the directions in the U. S. P. XIV (4). All *p*H values were determined with a Beckman *p*H Meter Model G.

A sample of acriflavine powder having a chloride content of 15.43 per cent, as determined by the N. F. method (1), was used as a standard. All other chemicals were reagent grade and were used without further purification.

Measuring the Curves.—In measuring the curves, the diffusion current was taken at the point of intersection of lines drawn through the slope and diffusion current portion of the wave. The residual current at the point of the measurement of the diffusion current was determined by extrapolation.

Polarographic Curves of Acriflavine.—The polarographic curves of acriflavine, under certain conditions, are well defined and have two waves. A plot of E *vs.* $\log i/id - i$ yields a straight line for both waves in acid and basic solutions. The slope of this line is close to the theoretical value for the reaction involving one electron for both waves in acid solution, and for the second wave only in alkaline solutions. In alkaline solutions, the plot of E *vs.* $\log i/id - i$ is a straight line, but a value of 1.8 for the slope of this line indicates that the electrode reaction is irreversible above *p*H 5.5. Since there is a break in the curve of the plot of $E_{1/2}$ *vs.* the *p*H of the solution at a *p*H of 5.5, for the first wave, the

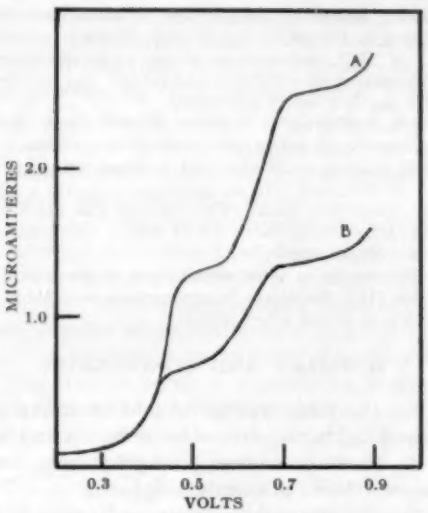

Fig. 1.—Typical curves of acriflavine polarographed in 0.1 *M* HCl. Curve *A*, 0.01%, curve *B*, 0.005% acriflavine

TABLE I.—EFFECT OF THE *p*H ON THE HALF-WAVE POTENTIAL AND DIFFUSION CURRENT OF ACRIFLAVINE[a]

	First Wave		Second Wave	
*p*H	$E_{1/2}$, Volt	*id*, Microamp.	$E_{1/2}$, Volt	*id*, Microamp.
1.1	0.445	0.92	0.645	0.92
1.6	0.500	0.90	0.680	0.83
3.0	0.570	0.89	0.800	0.83
5.1	0.710	0.89	0.960	0.75
6.2	0.750	0.67	[c]	[c]
6.8	0.750	0.77	[c]	[c]
7.0[b]	0.990	0.86	1.47	0.87
7.4	0.750	0.89	1.15	1.41
8.8	0.750	0.92	1.23	1.14
9.9[d]	0.800	0.95	1.31	1.10

[a] Concentrated acriflavine 0.01%.
[b] Unbuffered 0.1 *M* KCl as supporting electrolyte.
[c] No leveling off of the diffusion current.
[d] Supporting electrolyte 0.1 *M* KCl with NaOH to raise the *p*H.

electrode reaction apparently depends upon the form in which the acriflavine exists in solution, either as the salt or base.

Figure 1 shows some typical polarographic curves of acriflavine in 0.1 *M* HCl. In addition to 0.1 *M* HCl, KCl, $BaCl_2$, and $KC_2H_3O_2$ in 0.1 *M* concentrations were found to be satisfactory supporting electrolytes. The curves seem to be independent of the salt used as the supporting electrolyte, but very dependent upon the *p*H of the solution.

Effect of the *p*H on the Polarographic Curves of Acriflavine.—The polarographic reduction of acriflavine in buffered solutions results in one or two measurable waves, depending upon the *p*H of the solutions. The half-wave potential for the first wave, from *p*H 1.1 to 5.5, is calculated from the equation:

$$E_{1/2} = 0.39 \text{ v.} + 0.065\ p\text{H} \qquad (1)$$

Above *p*H 5.5, the half-wave potential is constant at 0.75 volt. The half-wave potential of the second wave is determined by the equation:

$$E_{1/2} = 0.59 \text{ v.} + 0.73\ p\text{H} \qquad (2)$$

from *p*H 1.1 to 8.8. The second wave did not have a well-defined break between the *p*H values of 5.5 and 7.0. In this region, the current increases indefinitely as the potential is increased. However, in unbuffered solutions, in this *p*H region, a break in the second wave does occur. The half-wave potentials of acriflavine in unbuffered solutions do not agree with those in buffered solutions. A summary of the effect of the *p*H of the solution on the half-wave potential and the diffusion current of acriflavine is shown in Table I.

Effect of the Concentration of Acriflavine on the Polarographic Curve of Acriflavine.—In acid solutions, *p*H 1.1, the half-wave potential becomes less negative as the concentration of acriflavine decreases. The half-wave potential for the first wave may be calculated from the equation:

$$E_{1/2} = 0.39 \text{ v.} + 4.8\ C \qquad (3)$$

and for the second wave:

$$E_{1/2} = 0.59 \text{ v.} + 4.8\ C \qquad (4)$$

where *C* is the concentration expressed in per cent. As the concentration increases, the relative heights of the first and second waves change, but the sum of the two diffusion currents is proportional to the concentrations. The ratio of the sum of the two diffusion currents expressed in microamperes, to the concentration expressed in per cent, is 180. The total height of the second wave as measured from the extrapolated residual current of the first wave is also proportional to the concentration. These data are summarized in Table II.

The data presented in Table II were used to prepare a calibration curve by plotting the total height of the second wave against the concentration of acriflavine.

TABLE II.—THE EFFECT OF THE CONCENTRATION OF ACRIFLAVINE ON THE POLAROGRAPHIC CURVE OF ACRIFLAVINE

	First Wave		Second Wave		Total Height of Second Wave[a]
Concn., %	$E_{1/2}$, Volt	*id*, Microamp.	$E_{1/2}$, Volt	*id*, Microamp.	
0.025	0.515	2.46	0.715	2.12	5.05
0.020	0.495	1.90	0.680	1.69	4.00
0.010	0.445	0.92	0.640	0.92	2.00
0.0050	0.415	0.41	0.615	0.46	1.04
0.0031	0.405	0.28	0.610	0.28	0.60

[a] Expressed in microamperes. Measured from residual current of solution.

TABLE III.—RESULTS OF THE DETERMINATION OF ACRIFLAVINE IN VARIOUS SAMPLES

Sample	Solutions, Calc. Amt.,[a] %	% Recovery
1	0.025	98.5
2	0.020	98.5
3	0.0125	100.0
4	0.00625	100.0
5	0.0031	97.0
6	0.010	102.0
7	0.025	98.5
	Triturates, Calc. Amt.,[a] Gm.	
1	0.20	99.0
2	0.15	98.6
3	0.05	97.0
	Tablets, Labeled Amt., Gm.	
1[b]	0.10	99.0
2	0.10	99.6
3	0.0655	98.2
	Ointment, Labeled Amt., %	
1	0.072	97.0

[a] Calculated from weighed amounts of acriflavine.
[b] Average of five determinations in duplicate on the same batch of tablets. Average deviation of the ten determinations was 0.3%.

Determination of Acriflavine.—Samples of acriflavine powder were dissolved in 0.1 *M* HCl and aliquots, representing various concentrations of acriflavine, were diluted with 0.1 *M* HCl and polarographed after removing oxygen as previously described. Powder triturates were made by carefully weighing portions of the standard acriflavine and mixing with lactose. The weight of acriflavine present in each triturate was not disclosed to the individual running the analysis, until after the determination had been completed. These samples were dissolved in 0.1 *M* HCl, diluted to give a concentration of approximately 0.025% acriflavine, and polarographed. The proper dilution could be judged from the color of the first solution.

Acriflavine in tablets was determined by weighing and powdering 20 tablets, taking an amount of powder equivalent to 0.1 Gm. of acriflavine, dissolving in 100 ml. of 0.1 *M* HCl, diluting 1:4 with 0.1 *M* HCl, and polarographing as in the above. The coating on enteric coated tablets was removed with acetone before powdering.

The acriflavine in only one ointment was determined. An amount of ointment equivalent to 0.025 Gm. of acriflavine was weighed, mixed with 0.1 *M* HCl, and filtered quantitatively into a 100-ml. volumetric flask. The volume was made up to exactly 100 ml. with 0.1 *M* HCl. This solution was then polarographed.

The results of these determinations are given in Table III. Duplicate determinations were made in each case except where noted.

SUMMARY AND CONCLUSIONS

1. The polarographic behavior of acriflavine in acid and basic solutions has been investigated.

2. Results show that, polarographically, acriflavine behaves as a single compound.

3. The electrode reaction is reversible in acid solution for both waves. In neutral and basic solutions, the electrode reaction is reversible only for the second wave.

4. Equations have been given to determine the half-wave potential in solutions of varying pH and concentrations of acriflavine.

5. The curves are well defined and may be used in the analytical determination of acriflavine.

6. The polarographic method for the determination of acriflavine is more rapid and more meaningful than the present N. F. method.

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Purification of Relaxin by Ion Exchange Resins*

By G. P. LEHRMAN, EGIL RAMSTAD, and M. X. ZARROW

The preparation of relaxin extracts from pregnant sow ovaries is described. Studies with ion exchange resins show that relaxin is a neutral substance. By the concomitant use of strong cationic and strong anionic ion exchange resins, a shortened extraction procedure was devised which allows the removal of most of the extraneous nitrogenous content of the relaxin extracts.

ONE OF THE OUTSTANDING MANIFESTATIONS of endocrine control of physiological functions is observed during pregnancy and parturition. It has been known for a long time that the sex steroids from the ovary and placenta and certain protein hormones from the pituitary and placenta are involved in this phenomenon. Recently Hisaw and Zarrow (1) have indicated that relaxin is also a hormone of pregnancy. Relaxin is a water-soluble extract of the ovary and

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possesses certain physiological activities which indicate a role in pregnancy. These are as follows: (*a*) relaxation of the pubic symphysis of certain mammals, (*b*) stimulation of the mammary gland, (*c*) relaxation of the uterine cervix. In addition this hormone appears in increasing amounts in the blood during pregnancy and is absent in the nonpregnant animal (1).

Hormonal preparations capable of inducing symphyseal relaxation have been made and studied rather extensively from a physiological viewpoint. To the present date, however, relaxin remains an impure extract and its true chemical structure is in the realm of conjecture. A high state of purity or a crystalline product is imperative for future valid physiological experimentation, and possible evaluation of this hormone as a therapeutic agent.

The purpose of this investigation is an attempt to simplify the methods of preparing purified relaxin extracts and to obtain higher purity of the relaxin preparations than have been previously available.

Early extraction procedures produced rather impure relaxin preparations. Hisaw, *et al.* (2), reported a preparation obtained by extracting *corpus luteum* tissue with acidulated alcohol. Further purification was effected by fractional precipitation from alcohol and acetone. Albert *et al.* (3), using corporal luteal residues, prepared a stable amorphous powder by extracting with acidulated alcohol in the presence of one molar sodium chloride followed by precipitation with ammonium sulfate. These workers (4) also prepared highly active relaxin extracts from fresh whole sow ovaries. The ovaries contained several hundred times the relaxin content of fresh or defatted *corpora lutea*. Their procedure consisted primarily in extracting the ovaries with acidified alcohol and sodium chloride followed by an adsorption of the active principle on a bentonite column. A very active eluate was obtained but yields were low due to losses during the concentration process. Frieden and Hisaw (5) have utilized isoelectric and alcoholic precipitation to prepare relaxin extracts of fairly high purity. They have shown saline solutions to be far superior to the acidulated alcohol as an extracting medium.

The methods proposed for extraction and purification of relaxin are elaborate. Relaxin shows behavior properties which suggest a compound of high molecular weight, possibly a polypetide. It would seem logical, therefore, to make use of ion exchange resins for the isolation of the hormone.

When active solutions of relaxin are passed through columns of a strong anionic resin (Amberlite® IRA-400) or a strong cationic resin (Amberlite® IR-120), the activity of the solution passes into the eluate, i. e., relaxin is not adsorbed on the resin. Relaxin, therefore, cannot contain free basic or acidic groups. Since proteins and polypetides in general contain ionizable groups, ion exchange resins should furnish a means of purifying relaxin preparations and of shortening the existing methods of preparation of relaxin concentrates. In fact, it was found that Amberlite® IRA-400 removed two-thirds of the nonactive nitrogenous content of crude alcoholic relaxin preparations and Amberlite® IR-120 removed two-thirds of the remaining nitrogenous content. The concomitant use of these resins reduced the nitrogen content of the alcoholic extracts by the factor of ten.

EXPERIMENTAL

Assay.—The relaxin activity of the various fractions was assayed by the biological method of Hisaw (6). This assay depends on the relaxation of the pubic symphysis of the guinea pig and is observed within six hours after administration of the hormone. Castrated female guinea pigs are used and the animals are primed with estradiol for four days prior to administration of the relaxin. Abramowitz (8) has quantitated the assay and expressed the results as guinea-pig units. A guinea pig unit (G. P. U.) is defined as that amount of relaxin which will induce symphyseal relaxation in two-thirds of a group of twelve guinea pigs weighing from 300 to 800 Gm. Thirty to 100 animals are needed for each assay.

Preparation of Relaxin Extracts.—The preparation of the crude relaxin extracts followed largely the method described by Frieden and Hisaw (5). Checks on the efficiency of the extraction showed the yield of relaxin to be small, and a modified method was employed for large-scale preparation of these extracts.

Fresh sow ovaries, a rich source of relaxin (7, 8), received in the frozen state from the packer, were reduced to a fine minced condition by means of a Hobart electric grinder. A total of 4,620 Gm. of the homogenate were placed in a 10-gallon stone jar and 18 liters of distilled water and 500 ml. of 36% hydrochloric acid were added. After maceration for two days at 7°, 750 Gm. of sodium chloride was stirred into the suspension. Undesired proteins were precipitated by adding 22 liters of 95% alcohol to the entire crude suspension. Separation of the solid matter was accomplished by screening and filtration through muslin. The hormone could be precipitated nearly quantitatively by saturating the alcoholic aqueous solution, at *p*H 5.4 (obtained by use of NaOH solution), with ammonium sulfate. The suspension was left for several days in the cold. The amorphous precipitate was collected and dissolved in cold distilled water. The solution was dialyzed against ice-cold distilled water until the dialyzate no longer gave a positive test with barium chloride solution. Some proteinaceous material

precipitated during the dialysis and was rejected. The crude relaxin was precipitated by pouring the solution (300 ml.) into 13 liters of cold acetone. The precipitate was redissolved in water and the solution used for assays and further purification studies.

Purification Studies with Ion Exchange Resins.—Relaxin solutions, prepared as described above, were passed in quantities of 10 ml. through 1 x 15-cm. columns of Amberlite® IR-120 (H) and IRA-400 (OH). The effluents were assayed biologically. Activity of influent: 125 G. P. U./ml.; activity of effluent: 100 G. P. U./ml.

Shortened Extraction Procedure.—An ovarian homogenate was extracted in the refrigerator twice for two days with 5% sodium chloride solution.

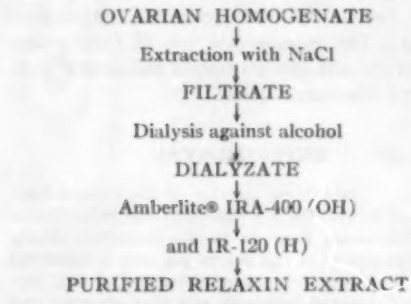

Fig. 1.—Shortened extraction procedure for preparation of relaxin.

Dialysis against an equal volume of alcohol precipitated much proteinaceous material, which was discarded. The alcoholic filtrate was passed through a column of Amberlite® IRA-400. The effluent was then passed through a column of Amberlite® IR-120. The alcoholic filtrate, the IRA-400 effluent, and the IR-120 effluent were bioassayed. Ninety guinea pigs were used in each test. Simultaneously, the alcoholic filtrate, the IRA-400 effluent, and the IR-120 effluent were subjected to Kjeldahl nitrogen determinations in order to establish the amount of nitrogen removed from the extracts by the resins employed in the purification. The results were as follows:

Fraction	Nitrogen Content/ ml.	Assay G. P. U./ml.	Potency G. P. U./ mg.
EtOH filtrate	1.0 mg.	200	200
IRA-400 effluent	0.35 mg.	200	600
IR-120 effluent	0.1 mg.	200	2000

SUMMARY

1. Relaxin extracts have been prepared from pregnant sow ovaries.

2. Relaxin is not adsorbed on a cationic exchange resin (Amberlite® IR-120).

3. Relaxin is not adsorbed on an anionic exchange resin (Amberlite® IRA-400).

4. A shortened extraction procedure for the preparation of relaxin has been introduced wherein ion exchange resins are used to eliminate nitrogenous compounds other than relaxin.

5. The nitrogen content of relaxin extracts prepared according to the best present methods has been reduced ten times by passage through columns of Amberlite® IRA-400 and Amberlite® IR-120.

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Pressor Drugs. III. Safety of Inhalational Therapy*

By JAMES C. MUNCH†

Administration of racemic epinephrine solution (Vaponefrin®) in quantities from 3 to 30 mg. daily for intervals up to eighty-eight weeks did not produce significant alterations in behavior, blood picture, weight gain, nor visceral changes in rabbits. Controlled experiments were conducted with U. S. P. Reference Standard Epinephrine and commercial solutions. These results agree with the data on humans in showing safety of inhalation therapy of racemic epinephrine solution under investigational conditions.

INHALATIONAL THERAPY has developed particularly within the last twenty years. Various types of drugs, previously administered by other methods, have proven therapeutically effective, not only in the treatment of pulmonary involvements but also for systemic action (1, 2, 3, 5, 11, 13, 15, 20–23). The size of inhaled par-

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ticles determines the nature of local, as well as systemic effects (6, 14, 19). If particles have a diameter of 30 microns or above, they tend to impinge on the tracheobronchial tree, may be swallowed, and may cause local irritation. If the particles have an average diameter below 0.5 micron, they tend to be exhaled without producing local or systemic effects. The ideal particle size appears to fall between 0.5 and 2.0 microns to produce local effects within the bronchioles or alveolar sacs. Investigations of sizes of particles delivered from various atomizers and nebulizers have shown great variations in particulate size, which would appear to be related to *effectiveness* of inhaled material on the one hand, and to the *safety* of inhaled material on the other hand.

Since the report of effectiveness of inhalation of epinephrine for the relief of asthmatic symptoms by Graeser and Rowe (13), there have been a number of reports confirming their findings (2). Most of the authors have indicated or suggested that there were no side-effects. Some reports have been observed suggesting untoward actions following prolonged inhalation, but in general fail to state the type of nebulizer or atomizer used, the *p*H and osmotic pressure of solution, frequency, duration, and local conditions of the tracheobronchial tissues at the time of exposure. These and other factors must be considered in connection with the problem of safety (1, 4, 7, 9, 10, 12, 16).

Studies on mice (16), rabbits (10, 12), cats (12), and humans (4, 12) indicated loss of tracheal cilia, irritation of the tracheal mucosa, and some desquamation of tracheal and bronchial epithelium. Segal, *et al.*, (24) have observed such changes in chronic bronchial asthma, especially with associated complications. It would appear that the *p*H was an important factor since Fabricant (9) produced substantially the same pathological changes in the respiratory nasal mucous membranes of rabbits by the administration of ephedrine hydrochloride solutions buffered to *p*H 3.0 to 4.5, 7.0 and 11.5 to 12.0. He points out that the *p*H of the secretion in clinically normal adult nasal passages ranges from 5.5 to 6.5, and in infants and young children from 5.0 to 6.7. During acute rhinitis, sinusitis, and the more active phases of allergic rhinitis, the *p*H changes to the basic side developing nasal alkalosis. With recovery the *p*H returns to the normal acid range.

Our previous studies on pressor drugs (17, 18) have dealt with the chemistry, pharmacology, and stability of *l*- and *r*-epinephrine solutions. Our unpublished studies have confirmed reports in the literature (1, 7, 12) that there is little or no systemic response following inhalation of nebulized epinephrine. No significant changes developed in the blood pressure of normotensive or hypertensive subjects, nor in the blood sugar of diabetics who received substantial quantities of 2.25 per cent racemic epinephrine solution (Vaponefrin®) from a standard Vaponefrin® nebulizer which delivered nebulized material within the optimal size of particles. Because of the continued and prolonged use of aerosol therapy with sympathomimetic amines and other drugs in the treatment of various types of bronchospasm, a series of investigations was planned to learn the safety of such therapy with Vaponefrin®, and one per cent *l*-epinephrine, using 0.9 per cent NaCl as a control. This report deals with results of studies on rabbits under laboratory conditions, supplementing an earlier communication (8). Similar studies on human subjects will be reported separately.

EXPERIMENTAL

A number of healthy Belgian hares of both sexes, from the same source, were maintained in individual cages on stock laboratory diets for a month or more before being selected for use. Growth was normal, so supplementation was not required. A number of rabbits of similar weight and condition and of both sexes were placed in four groups, and inhalations of test solutions nebulized from a standard glass Vaponefrin® nebulizer with a rubber hand bulb were given orally daily, five days per week, for intervals up to eighty-eight weeks. The nebulizers delivered 3 mg. of test solution per compression of the hand bulb. In the early part of the study, each rabbit was given 1 or 2 inhalations daily, orally, synchronized with inhalation by the rabbit; later the number of inhalations was increased to 10 daily without producing obvious effects. The greatest number of inhalations was 5,820 to Rabbit 3A, corresponding to 17.5 Gm. of solution, or over 400 mg. of *r*-epinephrine over a period of sixty weeks. The quantity of Vaponefrin® administered to each rabbit daily is equal to or greater than that which would be inhaled by a human in the same period of time. From time to time during the investigation, a rabbit was sacrificed for histopathological study. At the close of the eighty-eighth week all surviving rabbits were sacrificed. Blood examinations of all rabbits were made at the beginning, during, and at the close of the study; no departures from the accepted values for untreated rabbits were observed.

The duration of treatment and the total numbers of inhalations of each solution are recorded in Table I. Rabbits were weighed weekly at the same time of day, to determine any possible trend of change in weight. In the beginning, the body weights ranged from 1.33 to 2.5 Kg., with a modal value of 2.0 Kg. The average weekly gain in weight of rabbits receiving 0.9% NaCl was 0.9%; for rabbits receiving Vaponefrin®, 1.46%; for rabbits receiving freshly prepared 1% solution of U. S. P. Reference Standard *l*-Epinephrine as the hydrochloride, 1.42%;

TABLE I.—CHRONIC INHALATION STUDIES ON RABBITS. HISTOPATHOLOGICAL FINDINGS ON TISSUES

Rabbit No.	Weeks	Total Inhalations	Pulmonary Tissue: Trachea	Pulmonary Tissue: Bifurcation	Pulmonary Tissue: Alveoli	Stomach	Liver	Kidney	Adrenal	Cardiac Muscle
			(A) 0.9% NaCl							
14	19	835	WNL[a]	—[b]	4	—	—	WNL	—	—
15	31	1465	WNL	—	1	WNL	♯	1	WNL	—
13	43	2085	WNL	—	♯[c]	—	WNL	4	4	—
			(B) Vaponefrin Solution							
4	1	40	WNL	WNL	WNL	—	WNL	♯	—	—
3	8	58	WNL	—	WNL	—	—	1	—	—
16	3	140	WNL	♯	WNL	—	WNL	WNL	—	—
9	3	170	WNL	—	WNL	WNL	♯	♯	—	—
22	3	270	—	—	♯	—	2	3	—	—
2	31	297	WNL	WNL	♯	2	1	♯	WNL	—
1	88	777	WNL	WNL	WNL	—	2	♯	WNL	WNL
19	10	920	WNL	—	WNL	—	WNL	WNL	—	—
6	31	1470	WNL	—	♯	WNL	WNL	WNL	WNL	—
5	37	1750	WNL	WNL	♯	WNL	1	WNL	WNL	—
8	29	2750	WNL	WNL	1	—	♯	—	—	—
7	31	2970	—	WNL	♯	—	1	1	WNL	—
4B	72	2460	WNL	WNL	WNL	—	WNL	♯	WNL	WNL
3A	60	5820	WNL	WNL	1	—	♯	WNL	—	—
			(C) Reference Standard Epinephrine Solution 1:100							
25	2	100	—	—	WNL	—	WNL	—	—	—
24A	28	1380	3	WNL	WNL	WNL	WNL	WNL	WNL	—
22A	73	3610	WNL	WNL	♯	—	WNL	2	WNL	WNL
			(D) Commercial Epinephrine Solution 1:100							
11	3	85	WNL	—	WNL	—	1	WNL	—	—
10	31	1465	—	WNL	♯	WNL	♯	WNL	—	—
12	78	3845	WNL	WNL	WNL	—	♯	2	WNL	1

[a] WNL = Within Normal Limits.
[b] — = No section taken.
[c] ♯ = Pathology Slight; Numbers 1 to 4 show increasing pathology to maximal change.

and for a commercial 1% *l*-epinephrine hydrochloride, 1.92%. Because of the comparatively small number of animals completing the entire course of treatment, detailed statistical examinations are unwarranted, but there was no significant interference with gain in body weight which could be attributed to the treatment given.

At the conclusion of various intervals, one or more rabbits from each group were sacrificed. Gross examinations were made by the pathologists, and sections of the trachea, bifurcation and alveoli, the stomach, liver, kidney, adrenal and (in some rabbits) cardiac muscle were removed promptly and transferred to 4% formalin or to Boudin's fluid for fixation for microscopic study. No significant deviations from normal appearance were observed in the gross inspection of tissues. The fixed sections were imbedded, sectioned, and stained in the usual manner. Results of examination of the stained sections are recorded in Table I.

DISCUSSION

Our experience has indicated that there may be considerable variation in the histopathological appearance of tissues from "normal" rabbits. Therefore, we have used the term "Within Normal Limits (WNL)" to include such customary deviations. Slight departures from the normal limits are not considered as related to drug administration, unless increased dosage or prolonged duration of treatment produced greater degrees of the same type of alteration. Otherwise, such alterations were considered to be adventitious or agnogenic. Slight deviations from normal were recorded as "♯"; increasing pathologic alterations were denoted by numbers from "1" as slight to "4" as maximal. There was no evidence of histopathological alteration which could be attributed to inhalation of the test solution.

These results agree with the findings of Baldwin, *et al.* (1), and of Bullen (7) on humans. Baldwin and associates reported that several patients used Vaponefrin® for years without harm. Autopsies on two patients, who had used it continuously for four years and for nine years, revealed no pathology of the bronchial mucosa that could be ascribed to the drug. The authors concluded: "The daily inhalation of a bronchodilator must be considered harmless."

Bullen (7) conducted autopsies on 132 hospitalized asthmatic patients who died. In about one-third of the patients the cause of death was asthma. No serious hazard was presented to the heart unless emphysema occurred. Regarding epinephrine, he concludes: "The long-continued use of epinephrine by injection and inhalation produced no recognizable pathological changes in these patients."

Previous studies (17, 18) showed that the acute toxicity of Vaponefrin® was about two-thirds that of Reference Standard *l*-Epinephrine solution, following oral or intraperitoneal injection to animals. Giving essentially the same quantities of these two solutions to rabbits by inhalation, this investigation failed to determine any alterations of growth, behavior, or of the important viscera after administration for as long as eighty-eight weeks.

CONCLUSIONS

1. The inhalation of 3 to 30 mg. of racemic

epinephrine solution (Vaponefrin®) daily for periods as long as eighty-eight weeks did not produce significant alterations in behavior, in blood pictures, nor in rate of gain in body weight of rabbits.

2. Inhalations of Vaponefrin® up to 5,820 (corresponding to 400 mg. of the drug) did not produce detectable changes in the gross or the histopathological appearance of the importance viscera of rabbits.

3. These findings on rabbits agree with reports of autopsies on humans, in showing the safety of inhalation of *r*-epinephrine solution (Vaponefrin®) from a Vaponefrin® nebulizer over a substantial period of time.

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Application of the Hegman Gauge to Medicinal Particle Fineness in Ointments*

By G. J. LAFFERTY and H. M. GROSS

The Hegman Gauge was investigated as a manufacturing control device in the determination of fineness of medicinal particles dispersed in ointment bases. Results indicate the Hegman Gauge is readily used with a minimum of skill required to obtain results indicative of the state of subdivision of the dispersed particles. A correlation has been established between Hegman Gauge readings and microscopic measurements of the "width" dimension of the largest observable particle in an ointment. Further, a correlation is indicated between Hegman Gauge readings of ointments and the dimensional apertures applied to U. S. Standard Sieves used to classify the particles dispersed in the ointment.

IT HAS BEEN ESTABLISHED that the particle size of medicinals dispersed in ointment bases has a direct influence on tissue irritation, drug absorption, and the therapeutic efficacy of the medicament (1, 2). Various pharmaceutical texts and references advise initial levigation of medicinals and subsequent mixing and roller-mill finishing to produce homogeneous ointments with therapeutic constituents of small particle size (3, 4).

The physical characteristics of finished ointments have been studied and methods and devices for measuring viscosity or consistency of ointments have been originated (5, 6). The U. S. P. Penetrometer Test is an example of such an apparatus. Many methods of determining melting and pour points of ointments have been promulgated. However, to date only two methods for determining particle size of medicinals dispersed in ointment bases have been noted. Obviously, microscopic examinations for particle size are possible using calibrated attachments. In these examinations, ointments are thinned to proper fluidity either with an appropriate solvent to dissolve the base but not the dispersed medicinal or by addition of a fluid miscible with the base, and the particles are measured by examination of the prepared slide. Martens (7) described a method for determining particle-size distribution as applicable to ointments by calculation from the speed of fall of drug particles in a nonsolvent. The method depends on the density of the particle and is influenced by the sphericity of the particle. The greater the departure from a sphere, the greater the error in determination. This latter method is time consuming and intricate in manipulation. The

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microscopic method is more easily performed but is not particularly adapted to use in manufacturing areas by unskilled technicians. A cursory method of examination is to spread ointment by means of a spatula and examine the film formed for large particles.

In the paint industry a similar problem existed in the determination of fineness of particles in suspension. The earliest method for inspecting paint quality involved the art of knifing out a thin film and examining it for particle fineness. This required experience to predict final product quality, and agreement between testers was a matter of luck rather than skill (8). Subsequently, standards (9) were prepared for use as controls in the knifing test. Later a Fineness Gauge to be used in conjunction with these standards was introduced by the St. Louis Paint Club (10) and in 1938 the Hegman Gauge (11) was developed to eliminate dependence on standard reference samples.

The Hegman Gauge provides a classification method utilizing a diminishing aperture which indicates directly the particle size of solids dispersed in a liquid or semisolid phase. Adaptations of this fundamental design are used extensively in the paint industry. According to a gauge manufacturer,[1] "these gauges are now also used in the food line to check chocolates, mustards, peanut butter and also to check cosmetics, waxes, clays, inks, and shoe dressings."

It is logical that the pharmaceutical industry should investigate the use of the Hegman Gauge for "on-the-spot" particle fineness determinations of medicinals dispersed in ointment bases.

THE GAUGE AND ITS OPERATION

The fundamental feature is a wedge-shaped channel about 13 mm. wide and 153 mm. long which is cut in a hardened tool steel block. A linear scale ranging from 0 to 120 microns is etched at the appropriate channel depths. A scraper blade of hardened precision ground stainless steel is used in the determination (Fig 1).

Resting the gauge on a flat surface, a generous amount of test ointment is placed in the deep portion of the channel corresponding to the largest micron size reading. Holding the scraper blade vertically and at a right angle to the horizontal surface of the gauge, it is drawn along the length of the gauge with firm pressure spreading the test ointment along the channel to the shallow end. The gauge is immediately examined for streaks in the ointment-filled channel caused by particles whose dimension is greater than the distance between the scraper blade and the floor of the wedge-shaped channel. The point of origin of the streaks is considered as the point at which the linear micron scale reading is taken. The test is simple, quick, and reproducible.

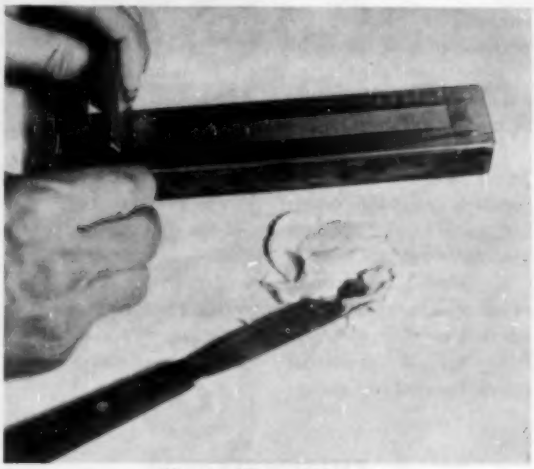

Fig. 1.—Hegman gauge.

This gauge and the description of its use are adaptations of the original Hegman Gauge and its method of use. A fineness gauge may be considered a continuous variable screen. It may be readily visualized that if a finely ground material were dispersed uniformly in a single particle thickness in the gauge path, some particles would protrude above the side of the channel beginning at the point that their physical measurement exceeded the depth of the channel. A straightedge moved slowly down the path to the shallow end would contact the protruding particles and on movement would screen or push these particles along the path forming the detectable streaks. The reading is made before confluence erases the streaks.

EXPERIMENTAL

Examination of Competitive Products.—Several competitive ointments prepared by dispersion of insoluble medicinal particles in an ointment base were examined microscopically to determine particle size of the medicinal. The microscopic slides were prepared by dilution of the ointments with liquid petrolatum. Hegman Gauge determinations were also made and reported as an average of ten tests. Results are recorded in Table I.

Application of Hegman Gauge Readings to Control of Processing.—Several of the competitive ointments examined exhibited a Hegman Gauge reading approximating 120 microns. Upon microscopic examination it was noted that the large particles causing this reading were clumps or aggregates of 5-micron particles. A portion of one of these ointments, No. 9, was triturated severely in a mortar and another was treated by muller and slab levigation.

Comparison of Hegman Gauge Readings and Microscopic Determination.—Nine batches of Whit-

[1] Precision Gauge Company, Dayton, Ohio.

field's Ointment N. F. were prepared from single lots of raw materials by various methods of preparation. Mortar and pestle, spatula and tile, spatula and ointment-paper pad, fusion and roller-mill methods of preparation were used. The prepared ointments were tested on the Hegman Gauge and reported as an average of 10 tests. The ointments were thinned with liquid petrolatum and examined microscopically to determine the size of the largest observed medicinal particle. The results are recorded in Table II.

Hegman Gauge Readings on Ointments Made from Screened Fractions.—Benzoic acid crystals were ground in a mortar and screen-classified through U. S. Standard Sieves. A 10% benzoic acid petrolatum ointment was prepared from each fraction. The fusion method of preparation was used to avoid reduction in particle size in preparation. Hegman Gauge determinations were made and reported as an average of 10 tests. A microscopic examination was made of the ointment fractions after dilution with liquid petrolatum and the largest observed particle noted. The results are recorded in Table III.

DISCUSSION OF RESULTS

The examination of competitive products indicated a wide range of control or lack of control of particle size between various manufacturers of the same product. Generally, the ophthalmic ointments were well prepared. Some products, for example Ointment No. 15, indicated the incorporation of original crystals of medicinal.

While the Hegman Gauge readings for each product did not correspond to the microscopic measurements, several factors account for the discrepancy. It should be understood that the method is not an accurate quantitative determination. It is designed as an indicator in the control of ointment manufacture. In some cases the Hegman Gauge indicated 120-micron particles in ointments which

TABLE I.—PARTICLE FINENESS DETERMINATIONS OF COMPETITIVE OINTMENTS BY HEGMAN GAUGE AND MICROSCOPIC EXAMINATION

Ointment Number	Type Ointment	Manufacturer	Hegman Gauge Readings,[a] Microns	Microscopic Measurement of Largest Particle, Microns	
				Width	Length
	Ophthalmic Ointments				
1	Boric Acid, U. S. P.	A	40	110	140
2	Ammoniated Mercury, U. S. P.	A	0	20	30
3	Yellow Mercuric Oxide, 1%, U. S. P.	A	0	5	5
4	Yellow Mercuric Oxide, 2 %	A	0	5	5
	Topical Ointments				
5	Zinc Oxide, U. S. P.	A	120	5[b]	5[b]
6	Zinc Oxide, U. S. P.	C	110	5[b]	5[b]
7	Zinc Oxide, U. S. P.	D	115	5[b]	5[b]
8	Sulfur, U. S. P.	A	50	40	65
9	Sulfur, U. S. P.	D	>120	5[b]	5[b]
10	Ammoniated Mercury, U. S. P.	A	30	5[b]	5[b]
11	Ammoniated Mercury, U. S. P.	C	60	5[b]	5[b]
12	Ammoniated Mercury, U. S. P.	D	120	5[b]	5[b]
13	Whitfield's, N. F.	A	60	95	225
14	Whitfield's, N. F.	B	85	160	390
15	Whitfield's, N. F.	C	>120	175	650
16	Boric Acid, U. S. P.	A	70	160	195
17	Boric Acid, U. S. P.	C	65	110	175
18	Boric Acid, U. S. P.	D	>120	160	225
19	Boric Acid, U. S. P.	E	65	100	175
20	Bacitracin Ointment, N. N. R.	CSC	0	30	40
21	Bacitracin-Neomycin, N. N. R.	CSC	0	30	40

[a] Denotes an average of 10 determinations.
[b] Denotes individual size of particles comprising aggregates.

TABLE II.—HEGMAN GAUGE READINGS COMPARED TO MICROSCOPIC EXAMINATION

Ointment Number	Material Used	Procedure for Ointment	Microscopic Measurement of Largest Particle, Microns		Hegman Gauge Readings,[a] Microns
			Width	Length	
22	Crude crystals	Spatula, on paper pad	120	715	120
23	Crude crystals	Mortar and pestle	390	680	>120
24	Crude crystals	Fusion + Mill (1x)	65	615	65
25	200 Mesh	Spatula, on paper pad	65	175	60
26	200 Mesh	Mortar and pestle	65	120	65
27	200 Mesh	Fusion	65	120	55
28	200 Mesh	Fusion + Mill (1x)	45	95	25
29	200 Mesh	Fusion + Mill (2x)	30	65	20
30	200 Mesh	Fusion + Mill (3x)	25	135	20

[a] Denotes an average of 10 determinations.

TABLE III.—HEGMAN GAUGE READINGS ON OINTMENTS MADE FROM SCREENED FRACTIONS

Ointment Number	U. S. Standard Sieve Fractions Through	On	Theoretical Screen Apertures, Microns	Hegman Gauge Readings,[a] Microns	Microscopic Measurement of Largest Particle, Microns Width	Length
31	120	140	125–105	85	130	450
32	140	200	105–74	70	125	450
33	200	230	74–62	65	75	150
34	230	270	62–53	60	70	150
35	270	325	53–44	55	70	140
36	325	...	<44	30	40	97

[a] Denotes an average of 10 determinations.

under microscopic examination exhibited 5-micron individual particles. However, these 5-micron particles were clumped to form the 120-micron offenders. Reasonably, the clump is as undesirable as the original crystal of the same size. Therefore, the Hegman Gauge was of advantage in its detection of clumped small particles. In other cases the Hegman Gauge readings were smaller than the microscopic measurement of the "width" dimension of the largest particles. It should be realized that the largest particle observed, whose dimensions are recorded in Table I, is not representative of the particle size of all the medicinal particles dispersed in the ointment base. In an average of 10 determinations by the Hegman Gauge, one determination of large magnitude is influenced by the other nine determinations which may not include a random large particle.

The portions of Ointment No. 9 which were triturated in a mortar or levigated by muller on a slab indicated Hegman Gauge readings of a lesser micron size after these attempts to reduce particle size. The readings were 85 and 80 microns, respectively, as compared to the greater than 120-micron original determinations indicating reduction in size of the clumps causing the high Hegman Gauge reading.

A comparison of Hegman Gauge readings and microscopic measurements of particles in ointments prepared from the same raw materials indicated a correlation between the Hegman Gauge reading and the "width" dimension of the microscopic particles observed. The ointments were prepared by various methods and the Hegman Gauge determinations indicated the effectiveness of particle-size diminution. The fact that the "length" dimension seemingly has no effect on the Hegman Gauge determination may be explained in the tendency for the needles to roll forward due to the motion of the scraper blade thus exposing the width dimension to form the detectable scratch.

Sieve-fractionated particles, corresponding to theoretical screen apertures, prepared into ointments exhibited a relative correlation between those dimensions and the Hegman Gauge reading. However, microscopic examination of the largest observable particles indicated particles whose "width" dimensions were greater than indicated by Hegman Gauge determinations. This discrepancy indicates the relative ineffectiveness of screening, in that occasional needle-like particles tend to pass on end through the sieves and result in extremely large-dimensioned crystals in fractions of lesser theoretical sieve size. It is felt that the Hegman Gauge reading is indicative of the particle size of the majority of the particles dispersed in the ointment.

SUMMARY

An adaptation of the Hegman Gauge has been applied to the determination of particle fineness of medicinals dispersed in ointments. Results indicate correlations between Hegman Gauge readings and the "width" dimension of dispersed particles as well as with particle fractions obtained through U.S. Standard Sieves.

The Hegman Gauge serves as a rapid method for production analysis and control of fineness of particles dispersed in ointments.

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A Spectrophotometric Assay for Aureomycin Hydrochloride*

By LOUIS J. RAVIN† and ARTHUR E. JAMES

A spectrophotometric method of assay involving the treatment of aureomycin hydrochloride with phosphomolybdic acid under controlled conditions has been developed. By use of this procedure, the aureomycin hydrochloride content of pharmaceutical products may be determined by comparison with a suitable reference standard.

IN VIEW OF THE FACT that the use of aureomycin hydrochloride as a therapeutic agent has greatly increased and that the current edition of the United States Pharmacopeia has not, as yet, adopted a method for its assay, it seemed relevant to investigate this problem.

Several methods of assay have been proposed. In 1949, Levine, Garlock, and Fischbach (1) published the details of a colorimetric assay for aureomycin hydrochloride measuring the absorbance of a hydrochloric acid solution at 440 mμ. In the same publication they reported a fluorometric assay method. Two years later, Hiscox (2) suggested a spectrophotometric assay for aureomycin hydrochloride measuring the absorbance of a sulfuric acid solution at 274 mμ and at 250 mμ. Sideri and Osol (3) developed an assay for aureomycin hydrochloride by titrating in a nonaqueous solvent.

In the preliminary stages of this study it was found that aureomycin hydrochloride on treatment with phosphomolybdic acid and sodium hydroxide developed a bluish-green color. It appears that this color is due to the reduction of the phosphomolybdic acid. Irrespective of the mechanism involved or the nature of the products, the amount of the reduction product developed was found to be proportional to the concentration of the aureomycin present. This fact is the basis for the spectrophotometric assay of aureomycin hydrochloride presented herewith.

EXPERIMENTAL

Color Maxima and *p*H.—Measurements were made keeping the concentration of aureomycin and of phosphomolybdic acid solution constant and varying the *p*H by addition of sodium hydroxide. It was found that the color was most intense between *p*H 5.5 and 6.5. Below *p*H 5.5, the color was not fully developed and above *p*H 7.0, it rapidly disappeared upon standing. In the subsequent work the colored solutions were kept between *p*H 5.5 and 6.5.

Color Maxima and Concentration of Phosphomolybdic Acid Solution.—The concentration of aureomycin hydrochloride and the *p*H of the solution were kept constant while the amount of the phosphomolybdic acid solution was varied. However, there appeared to be no noticeable change in the color intensity when volumes of the 2% phosphomolybdic acid solution were varied between 20 and 50 ml. Thus, it appears that at the lowest concentration of phosphomolybdic acid it was still in excess of the minimum amount required to react with the aureomycin. In the work that follows the volume of 2% phosphomolybdic acid was kept at 20 ml.

Determination of Absorption Maximum.—One-tenth of a gram of pure aureomycin hydrochloride[1] was dissolved in distilled water in a 1000-ml. volumetric flask. Twenty milliliters of a 2% solution of phosphomolybdic acid (Merck & Co., Inc., Reagent, 99.85%) was added. Ten per cent sodium hydroxide was added dropwise until the solution attained a *p*H of 5.5 to 6.5. The solution was brought to volume with distilled water. An aliquot portion showed that the *p*H remained less than 6.5.

The transmittancy spectrum was measured between 400 and 800 mμ by use of matched cuvettes.[2] A solution containing everything except aureomycin hydrochloride was used as the blank.

Figure 1 shows the data obtained, transcribed to graphical form. It is evident that the region of maximum absorbancy is between 405 and 415 mμ.

Beer's Law.—Various dilutions of the aureomycin hydrochloride standard solution were prepared to study its conformance to Beer's Law. Aliquot portions containing 0.0151 to 0.0402 mg., prepared from the aureomycin standard solution, were measured into 100-ml. volumetric flasks and brought to the mark with the blank solution. The absorbance of each solution was measured at 405 mμ. A straight line was obtained when the values for absorbance were plotted against the concentrations. In subsequent work, this line served as the standard reference graph in determining the aureomycin content of various pharmaceutical products.

The results of the assays are listed in the following table.

* Received August 27, 1954, Temple University, School of Pharmacy, Philadelphia, Pa.

Abstracted from a thesis presented to the Graduate School of Temple University, School of Pharmacy, by Louis J. Ravin in partial fulfillment of the requirements for the degree of Master of Science.

† Present address: University of Wisconsin, Madison, Wis.

Presented to the Scientific Section, A. PH. A., Boston meeting, August, 1954.

[1] Obtained through the courtesy of the Lederle Laboratories, Division of the American Cyanamid Company.

[2] A Coleman Universal Spectrophotometer, Model 14, was used to measure the transmittancy.

TABLE I.—AUREOMYCIN CONTENT OF VARIOUS PHARMACEUTICAL PRODUCTS

Product	Weight of Sample, Gm.	Found,[a] mg./1000 ml.	Labeled Content,
50-mg. tablets	0.0501	26.62	25.97
	0.0484	26.33	25.6
	0.0494	26.50	25.46
Surgical powder	0.1252	26.17	25.03
	0.1253	26.25	25.07
Triple sulfas tablets	0.1497	26.32	25.21
	0.1498	26.70	25.23
	0.1497	26.34	25.23
250-mg. capsules	0.0698	32.43	30.92
	0.0701	33.07	31.03
	0.0699	32.95	31.01
Ointment	0.8671	25.22	25.75
	0.8335	24.65	25.00

[a] Average of 3 determinations.

Assay of Pharmaceutical Products.—Twenty aureomycin hydrochloride soluble tablets were weighed and reduced to a fine powder. An aliquot portion of this powder, containing approximately 25 mg. of aureomycin hydrochloride, was weighed and transferred to a 1000-ml. volumetric flask. The aureomycin was dissolved in 150 ml. of distilled water. Twenty milliliters of the phosphomolybdic acid solution was added. The *p*H of the solution was increased to 5.5–6.5 by the addition of sodium hydroxide and brought to volume with distilled water. The absorbance of the colored solution produced was measured in the spectrophotometer set at 405 mμ. The concentration of the aureomycin hydrochloride corresponding to the measured absorbance was found by reference to the standard graph.

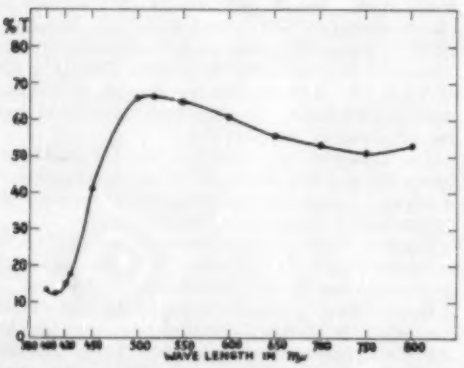

Fig. 1.—Absorption spectrum of aureomycin HCl.

An aliquot portion of Aureomycin Surgical Powder containing 25 mg. of aureomycin hydrochloride was weighed. The powder was transferred to a flask containing 150 ml. of distilled water. The solution was shaken intermittently for fifteen minutes to insure complete solution of the aureomycin. It was then filtered through Whatman No. 40 filter paper into a 1000-ml. volumetric flask. The residue on the filter paper was washed with small portions of distilled water. This solution was treated as previously described with phosphomolybdic acid, sodium hydroxide, and distilled water. The absorbance of the solution was then measured and the concentration of aureomycin determined by reference to the standard graph.

The contents of ten aureomycin hydrochloride capsules were weighed. An aliquot portion containing approximately 25 mg. of aureomycin hydrochloride was weighed and transferred to a flask containing 150 ml. of distilled water. The solution was treated as previously described under the procedure for aureomycin surgical powder. The absorbance of the colored solution was measured at 405 mμ and the concentration of the aureomycin determined by reference to the standard graph.

Ten tablets labeled Aureomycin Triple Sulfas[a] were weighed and reduced to a fine powder. An aliquot portion containing approximately 25 mg. of aureomycin hydrochloride was weighed and transferred to a flask containing 150 ml. of distilled water. The solution was treated as previously described under the procedure for aureomycin surgical powder.

An aliquot portion of aureomycin hydrochloride ointment containing approximately 25 mg. of aureomycin hydrochloride was weighed. It was transferred to a 250-ml. beaker containing 150 ml. of ether. The ointment base dissolved in the ether while the aureomycin, which is insoluble in this solvent, remained suspended in the ether. The ethereal solution was transferred to a separatory funnel containing 100 ml. of distilled water. The solution was shaken repeatedly for five minutes, and then the aqueous solution containing the aureomycin was withdrawn. The ethereal solution was shaken with two additional 100-ml. portions of distilled water to insure complete extraction of the aureomycin. The aqueous extracts were combined and heated on a water bath to drive off the dissolved ether. The solution was transferred to a 1000-ml. volumetric flask and treated as previously described under the procedure for Aureomycin Surgical Powder.

As an additional check on the validity of the phosphomolybdate method, the previously mentioned fluorometric method of assay as developed by Levine and his associates (1) was used to determine the aureomycin content of two of the products. Excellent agreement between the two methods was found.

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[a] Aureomycin Triple Sulfas contain: aureomycin hydrochloride, 0.125 Gm.; sulfadiazine, 0.167 Gm.; sulfamerazine, 0.167 Gm.; sulfamethazine, 0.167 Gm.

A Potentiometric Nonaqueous Assay for Barbiturates and Their Preparations*

By CHARLES J. SWARTZ† and NOEL E. FOSS

A quantitative study has been conducted on a series of barbiturates in order to ascertain if a suitable nonaqueous potentiometric method could be utilized for purposes of routine control of these drugs and their pharmaceutical preparations. The barbiturates selected are those which will be admitted into U. S. P. XV and N. F. X. The investigation also has included pharmaceutical dosage forms such as the tablet, elixir, ampul, capsule, and suppository. The method developed consists of a chloroform-polyethylene glycol 400 solvent system using sodium methoxide in absolute methanol as titrant. The procedure was found to be rapid, highly accurate, simple, and relatively inexpensive and it is suggested that it may be suitable for the routine control of pharmaceuticals in industry.

IN THE LAST DECADE ever-increasing emphasis has been placed on instrumental analysis as a routine control measure for the determination of the active ingredient in pharmaceutical preparations. The development of the potentiometric method in an aqueous medium provided a simple rapid method of assay for water-soluble constituents. Many of the new pharmaceuticals contain synthetic organic compounds which are not soluble in an aqueous medium and for these medicinals methods are being developed utilizing potentiometry in nonaqueous mediums. A number of surveys have appeared in the literature, covering various aspects of titrations in nonaqueous solutions, especially as applied to pharmaceuticals (1–4).

Nonaqueous titrimetric assays for acid-base systems possess the basic requirements for good analytical methods of speed, accuracy, and simplicity (5). These are primary requirements in a control laboratory and for specialized analyses.

Vespe and Fritz (6) as well as Green (7) titrated barbiturates in nonaqueous systems. Phenobarbital in polyethylene glycol (PEG) 400 solution was determined by Autian and Allen (8). Ryan, Yanowski, and Pifer also have made valuable contributions to this work (9).

This study was undertaken in order to ascertain whether the method developed by Autian and Allen for phenobarbital in PEG 400 solution could be applied with some modifications as a general assay procedure for barbiturates.

* Received September 25, 1954, from the University of Maryland, School of Pharmacy, Baltimore.
Presented to the Scientific Section, A. PH. A., Boston meeting, August, 1954.
Abstracted in part from a thesis submitted by Charles J. Swartz to the Graduate Council of the University of Maryland in partial fulfillment of the requirements for the degree of Master of Science.
† H. A. B. Duming Research Fellow, 1954.
Appreciation is expressed to the following for their generous grants of barbiturate samples: Abbott Laboratories, Ciba Pharmaceutical Products, Inc., Gane's Chemical Works, Hoffmann-La Roche, Inc., Eli Lilly and Company, Sandoz Chemical Works, and Winthrop-Stearns, Inc.

EXPERIMENTAL

The materials used were of reagent grade except where otherwise specified.

The solvent employed throughout was a mixture of chloroform and polyethylene glycol 400 U. S. P. A series of assays was carried out, employing amobarbital, using various percentages of these solvents to determine if there were any appreciable changes in the sensitivity of the end point by varying amounts of PEG 400. The percentages taken were 5, 10, 20, 40, and 50 % v/v, respectively, the remainder of the solvent being chloroform. The end point breaks as shown in Fig. 1 clearly demonstrated that 10% PEG 400 was the optimum concentration and this ratio was used throughout the remainder of the proceedings.

Attempts were made to use absolute ethyl alcohol in combination with chloroform as a solvent but preliminary studies along this approach were unsatisfactory.

The titrant employed was 0.1 *N* sodium methoxide in absolute methanol. Freshly cut sodium metal was dissolved in methanol, the solution filtered and stored in the reservoir of an automatic delivering buret. The titrant was standardized against benzoic acid dissolved in dimethylformamide with end points being determined visually using thymol blue as indicator.

A Fisher Titrimeter was utilized for most of the investigative work and a standard calomel-glass electrode system was employed satisfactorily throughout.

The extraction apparatus devised by Schmall (10) was used for the chloroform extraction of the acids from water solution or suspension of the metal salts of the barbiturates. This apparatus permits semiautomatic extraction with a clean separation of the solvent phase, thus assuring sharper nonaqueous end points, since water is not carried over with the organic solvent.

The "second derivative" method of Kolthoff and Furman (11) was employed for most end point determinations.

Assay Procedure.—The powdered barbiturate sample to be assayed, if it is the free acid, is dried for four hours at 80°. A 0.1 to 0.2-Gm. sample is accurately weighed, placed in a beaker, and exactly 5 cc. of polyethylene glycol 400 is added from a buret. The addition of 45 cc. of chloroform facilitates the

solution of the sample. The solution is then titrated with 0.1 *N* sodium methoxide utilizing the Fisher Titrimeter.

The powdered metal salts of the barbiturates are first dissolved in 10 cc. of water and extracted with chloroform, after acidification, in the "Schmall apparatus." After the extracted free acid solution is concentrated to 45 cc., PEG 400 is added and the titration carried on as previously mentioned.

The barbiturates selected for assay were those which have been proposed for admission in U. S. P. XV and N. F. X with the exception of phenobarbital which was included, although already official, as a result of its widespread usage and appearance in innumerable pharmaceuticals.

Ten samples of each pure barbiturate were taken and results are summarized in Table I.

TABLE I.—DETERMINATION OF PURE BARBITURATES

Compound	No. of Samples Taken	Average % Recovery
Allylbarbituric acid	10	99.9 ± 0.2
Allylisopropylbarbituric acid	10	99.8 ± 0.1
Amobarbital	10	100.1 ± 0.2
Butethal	10	100.2 ± 0.3
Cyclobarbital calcium	10	99.2 ± 0.2
Diallylbarbituric acid	10	100.0 ± 0.2
Hexobarbital	10	99.8 ± 0.1
Mephobarbital	10	99.7 ± 0.2
Phenobarbital	10	99.9 ± 0.3
Secobarbital sodium	10	99.6 ± 0.2

Pharmaceutical dosage forms of some of the barbiturates were assayed to determine the applicability of the method to control procedures in pharmaceutical manufacture. Five products were assayed and the results are summarized in Table II.

TABLE II.—DETERMINATION OF DOSAGE FORMS OF BARBITURATES

Sample	No. of Samples Taken	% of Labeled Concn. Found
Secobarbital sodium capsules	10	98.3 ± 0.2
Secobarbital sodium ampuls	10	99.7 ± 0.1
Secobarbital sodium suppositories	10	95.9 ± 0.7
Secobarbital elixir	10	104.2 ± 0.4
Amobarbital tablets	10	100.4 ± 0.2

DISCUSSION

It had originally been intended to use chloroform alone as a solvent but accurate potential readings could not be obtained with this nonionizing solvent. Placing the electrodes in a solution of chloroform immediately polarized them. Another factor which decrees against the use of chloroform alone as a solvent was that as soon as the sodium methoxide titrant was added to the solution to be assayed, a flocculent precipitate of the sodium barbiturate would appear. Thus the addition of a small amount of a lower polar alcohol is of value for more than just the reason of increasing the sensitivity of the end point.

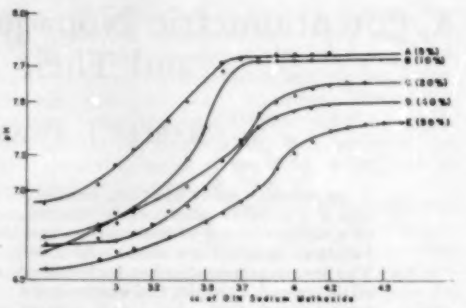

Fig. 1.—Effects of various percentages of polyethylene glycol 400 on sensitivity of end point. Drug—Amobarbital

All assays attempted proceeded smoothly with a minimum of difficulty being encountered in the manipulations. Special methods were adopted only in the case of cyclobarbital calcium powder which was not immediately soluble in water. An aqueous suspension was made of this powder and it was carried along as previously stated under the general method for barbiturate salts.

CONCLUSIONS

1. The method set forth is a rapid, accurate, simple, and relatively inexpensive method for the determination of barbiturates and their preparations.

2. The procedure is convenient and more rapid than the methods for most of the official barbiturates.

3. Determination of pharmaceutical dosage forms has responded with almost the same degree of precision and simplicity as the determination of powdered samples of pure drugs.

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Studies on Antacids. V. The Preparation and Properties of Dihydroxy Aluminum Sodium Carbonate*

By I. W. GROTE, J. M. HOLBERT, and MARJORIE FOX

The new compound, dihydroxy aluminum sodium carbonate, has been prepared and its properties as a possible gastric antacid studied. As could be expected from the formula, this water-insoluble, almost tasteless compound has both prompt and prolonged acid neutralizing properties when tested both *in vitro* and *in vivo*.

IN THE FIRST TWO PAPERS of this series (1, 2) we were primarily concerned with the development of an *in vitro* test designed to predict more accurately the clinical efficacy of antacid materials and to study in detail the antacid properties of commerically available antacids with particular attention to dihydroxy aluminum aminoacetate developed by Krantz, *et al.* (3). It had been previously shown by Rossett and Flexner (4) that the *in vitro* performance of an antacid closely parallels its clinical result. Since that time this work has been extended by such investigators as Hammarlund and Rising (5), Rossett and Rice (6), Murphey (7), Armstrong and Martin (8), Gore, Martin, and Taylor (9), Corrente (10), and others.

In the third paper of this series (11) we reported the preparation and properties of several basic aluminum derivatives of glutamic acid. In the preparation of dihydroxy aluminum aminoacetate and, for example, tetrahydroxydialuminum glutamate, liquid aluminum isopropylate was reacted with an aqueous solution of the appropriate amino acid. Under such conditions the OH ion concentration of the solution is depressed due to the acidity of the amino acid and the possibility of the formation of aluminum hydroxide correspondingly decreased. In our present investigation the situation was actually reversed and aluminum isopropylate was reacted with a basic solution of sodium bicarbonate. One might reasonably predict that due to the increased OH ion concentration the major reaction would be the formation of aluminum hydroxide. Such, however, was not the case and the reaction proceeded smoothly to give good yields of dihydroxy aluminum sodium carbonate. Although the mode of formation of the compound may be far more complex, it is thought that the reaction is best represented by the following equation:

$$Al[(CH_3)_2CHO]_3 + NaHCO_3 + 2H_2O \rightarrow (HO)_2AlOCO_2Na + 3(CH_3)_2CHOH$$

Dihydroxy aluminum sodium carbonate is a white, rather fluffy solid almost devoid of taste. It is insoluble in water and the usual organic solvents but dissolves readily in dilute acid with the release of carbon dioxide. As its formula would suggest, dihydroxy aluminum sodium carbonate possesses many of the properties of both sodium bicarbonate and aluminum hydroxide. Like sodium bicarbonate it neutralizes acid rapidly with the release of carbon dioxide but unlike sodium bicarbonate does not result in a *p*H on the alkaline side. It also exhibits the prolonged neutralizing power and buffering action of aluminum hydroxide gel.

The prolonged neutralization characteristics of dihydroxy aluminum sodium carbonate were determined by the method of Holbert, Noble, and Grote (2) with the results shown in Table I and Fig. 1.

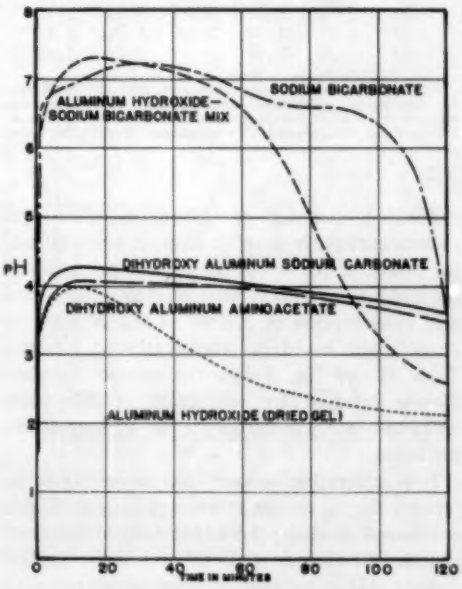

Fig. 1.—Prolonged neutralization

* Received August 27, 1954, from the Research Laboratory of Brayten Pharmaceutical Company, Chattanooga 9, Tenn. Presented to the Scientific Section, A. PH. A., Boston meeting, August, 1954.

It is readily seen that the compound produces a rapid neutralization to a *p*H of approximately 4 and maintains a *p*H of above 3 for at least two hours under the conditions of the test. On this basis one would predict that the oral ingestion of dihydroxy aluminum sodium carbonate by humans would lead to prompt and prolonged buffering of gastric acid. Clinical results to date have borne out this prediction. For the sake of comparison the prolonged neutralization curves of the dihydroxy aluminum aminoacetate, sodium bicarbonate and dried aluminum hydroxide gel and an equimolar mixture of sodium bicarbonate and dried aluminum hydroxide gel are also given in Table I and Fig. 1. From the data in Table I, Column III, and its corresponding curve, it is evident that dihydroxy aluminum sodium carbonate is not a mixture.

TABLE I.—PROLONGED BUFFERING ACTION OF ANTACID MATERIALS[a]

Time, Min.	*p*H				
	I	II	III	IV	V
0	1.60	1.64	1.61	1.61	1.64
1	3.89	3.02	6.47	6.68	3.18
3	4.09	3.70	6.59	6.70	3.61
5	4.12	3.89	6.78	6.81	3.78
7	4.20	3.98	6.94	6.86	3.82
10	4.20	4.03	7.18	6.97	3.92
20	4.20	4.02	7.29	7.10	3.81
30	4.19	4.02	7.21	7.22	3.51
40	4.14	4.02	7.04	7.19	3.22
50	4.08	3.99	6.92	7.10	2.89
60	3.99	3.96	6.55	6.84	2.67
70	3.92	3.89	6.05	6.67	2.49
80	3.88	3.83	5.09	6.60	2.31
90	3.83	3.75	3.99	6.56	2.26
100	3.74	3.68	3.30	6.32	2.19
110	3.64	3.57	2.82	5.70	2.11
120	3.58	3.49	...	3.22	2.09

[a] Column I—dihydroxy aluminum sodium carbonate; II—dihydroxy aluminum aminoacetate; III—equimolar mixture of sodium bicarbonate and aluminum hydroxide; IV—sodium bicarbonate; V—aluminum hydroxide (dried gel).

Purified samples of dihydroxy aluminum sodium carbonate show an average acid-consuming power of 267 cc. of 0.1 *N* acid per gram on the dry basis. This is contrasted to a theoretical acid consumption of 278 cc. of 0.1 *N* acid per gram when based on the suggested formula. Table II and Fig. 2 show the volume of carbon dioxide released by one gram of dihydroxy aluminum sodium carbonate at various acidic *p*H levels.

It is interesting to note that as acidity is increased the maximum volume of carbon dioxide is released at about the buffer zone of the compound. In clinical trials this release of carbon dioxide aids in promoting relief associated with eructation.

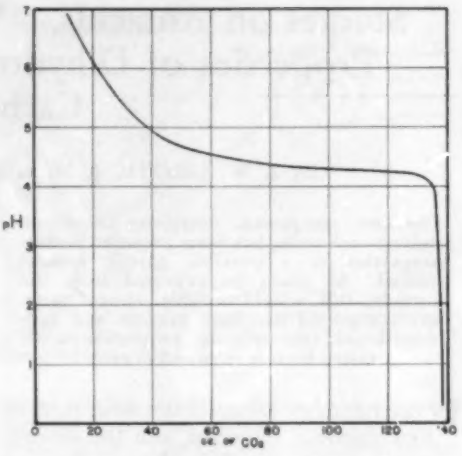

Fig. 2.—Cubic centimeters of CO_2 released from one gram of dihydroxy aluminum sodium carbonate

TABLE II.—VOLUME OF CARBON DIOXIDE RELEASED BY ONE GRAM OF DIHYDROXY ALUMINUM SODIUM CARBONATE

*p*H	cc. CO_2
6.91	11.2
5.55	25.6
4.87	44.8
4.58	55.8
4.48	60.4
4.46	67.4
4.36	85.0
4.31	97.0
4.28	116.0
4.17	130.0
4.22	131.0
1.47	137.0
1.12	136.0

EXPERIMENTAL

Dihydroxy Aluminum Sodium Carbonate—Sodium bicarbonate (42 Gm., 0.5 *M*) was dissolved in 350 cc. of warm water and stirred vigorously during the addition of liquid aluminum isopropylate (102 Gm., 0.5 *M*). A gelatinous precipitate formed rapidly which on continued stirring became granular. After filtering, the material was washed with water to remove any traces of sodium bicarbonate and dried to a constant weight. The yield was almost quantitative.

Anal.—Calcd. for CH_2O_3AlNa: Al, 19.0; CO_2, 30.6. Found: Al, 18.0; CO_2, 30.0.

PHARMACOLOGY

Acute toxicity studies on dihydroxy aluminum sodium carbonate show that when the compound was administered orally in 1.0% tragacanth suspension to female rats the LD_{50} was greater than 2.0 Gm./Kg. of body weight. Under similar conditions the LD_{50} for female mice was greater than 8.4 Gm./Kg. of body weight. These figures represent the largest dose that can be given mechanically under the conditions of the experiment. Out of 40 test animals, only one died.

In the chronic feeding studies on male and female albino rats, the animals were fed at levels of 0.1, 0.5, and 1.0% for thirteen weeks. None of the test animals showed any evidence of systemic toxicity nor did hematological and histological studies reveal any abnormalities in the test groups. Female mongrel dogs kept on high but sublethal doses of dihydroxy aluminum sodium carbonate for a period of two weeks showed no gross signs of systemic toxicity and plasma and urine studies did not reflect any appreciable change in the acid-base balance.

SUMMARY

1. The preparation and properties of a new antacid, dihydroxy aluminum sodium carbonate, are reported. This material combines many of the desirable features of the different types of antacids and shows both rapid and prolonged acid-neutralizing power.

2. Toxicity studies indicate that for all practical purposes dihydroxy aluminum sodium carbonate is nontoxic and does not appreciably alter the acid-base balance even in large doses.

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Essential Elimination of Sodium Cycloheptenylethylbarbiturate (Medomin®)[1] in Rabbits*

By THEODORE KOPPANYI, CHARLES F. MORGAN, and JOSEPH V. PRINCIOTTO

The essential elimination of Nembutal® sodium and Medomin® sodium was determined in albino rabbits and the results compared. The experimental animals eliminated about one-fourth of the LD_{50} per hour of Nembutal® and over one-third of the LD_{50} per hour of Medomin®. The average survival time after half-hourly intravenous administration of the LD_{50} was seven and one-half hours for Nembutal® and over nine and one-half hours for Medomin®. The results also show clearly that Medomin® is a significantly shorter acting barbiturate than Nembutal®.

THE ESSENTIAL ELIMINATION of barbiturates was first studied by Koppanyi and Lieberson (1) and subsequently by Hazleton, Koppanyi, and Linegar (2). Total essential elimination is equivalent to complete cessation of drug action, irrespective of whether the drug is excreted, metabolized, or stored in an inactive form in some tissues. The study of essential elimination, therefore, yields reliable figures as to the duration or persistence of drug action, no matter how the drug is cleared or inactivated.

EXPERIMENTAL

Albino rabbits were used for these experiments and aqueous solutions of the drugs were injected into the marginal ear vein. Twenty per cent (one-fifth) of the LD_{50} of pentobarbital sodium (Nembutal® sodium) and of cycloheptenylethylbarbiturate sodium (Medomin® sodium) was injected every half hour. Fifteen rabbits were given pentobarbital sodium, and fifteen were given Medomin®

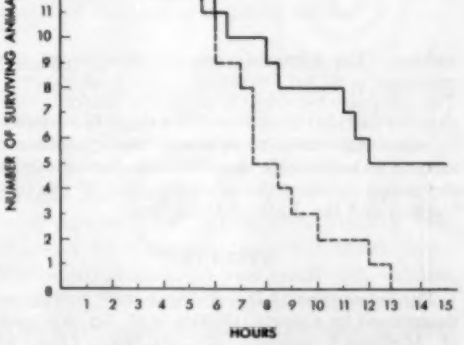

Fig. 1.—Results of injection of 8 mg./Kg. of Nembutal® sodium and 24 mg./Kg. of Medomin® sodium at half-hour intervals.

* Received August 16, 1954 from the Departments of Pharmacology and Physiology, Georgetown University Medical Center, Washington, D. C.
[1] Medomin® was supplied through the courtesy of Geigy Pharmaceuticals, New York, N. Y.

TABLE I.—NEMBUTAL®

Rabbit No.	Single Dose[a] mg/Kg.	No. of Doses	Interval Between Doses in minutes	Results	Total Dose Given in mg/Kg.	Total Dose in % of 50% Fatal Dose	Duration in minutes	Percentage of Essential Elimination of 50% Fatal Dose
1	8	15	30	D[b]	120.0	300.	450	200.
2	8	26	30	D	208.0	520.	780	420.
3	8	17	30	D	136.0	321.	510	240.
4	8	8	30	D	88.0	220.	240	120.
5	8	20	30	D	160.0	400.	600	300.
6	8	15	30	D	120.0	300.	450	200.
7	8	24	30	D	192.0	480.	720	380.
8	8	11	30	D	88.0	220.	330	120.
9	8	15	30	D	120.0	300.	450	200.
10	8	18	30	D	144.0	360.	540	260.
11	8	14	30	D	112.0	280.	420	180.
12	8	10	30	D	80.0	200.	300	100.
13	8	11	30	D	88.0	220.	240	120.
14	8	12	30	D	96.0	240.	360	140.
15	8	12	30	D	96.0	240.	360	140.
						Av. hr.	7.5	27.7 %/hr.

[a] Dose—1/5 of LD_{50}.
[b] D—Death.

TABLE II.—MEDOMIN®

Rabbit No.	Single Dose[a] mg/Kg.	No. of Doses	Interval Between Doses in minutes	Results	Total Dose Given in mg/Kg.	Total Dose in % of 50% Fatal Dose	Duration in minutes	Percentage of Essential Elimination of 50% Fatal Dose
1	24	28	30	R[b]	672.0	560.	840	>460.
2	24	7	30	D[c]	168.0	140.	210	400.
3	24	17	30	D	408.0	340.	510	240.
4	24	7	30	D	168.0	140.	210	400.
5	24	24	30	D	576.0	480.	720	382.
6	24	28	30	R	672.0	560.	840	>460.
7	24	13	30	D	312.0	260.	390	160.
8	24	23	30	D	552.0	460.	690	360.
9	24	16	30	D	384.0	320.	480	220.
10	24	12	30	D	288.0	240.	360	140.
11	24	28	30	R	672.0	560.	840	>460.
12	24	28	30	R	672.0	560.	840	>460.
13	24	22	30	D	528.0	440.	660	340.
14	24	28	30	R	672.0	560.	840	>460.
15	24	6	30	D	144.0	120.	180	200.
						Av. hr.	9.57	Av. is >35.7 %/hr.

[a] Dose—1/5 of LD_{50}.
[b] R—Recovery.
[c] D—Death.

sodium. The administration of these drugs was continued in all but five rabbits until death occurred. The difference between the fatal dose determined in this way and that determined by a single intravenous injection was taken to represent the approximate amount of barbiturate essentially eliminated during the period between the administration of the first fraction and the death of the animal.

RESULTS

The intravenous LD_{50} of Nembutal® sodium as determined by a single injection is 40 mg./Kg., and of Medomin® sodium, 120 mg./Kg. Thus, at half-hour intervals, 8 mg./Kg. of the first barbiturate, and 24 mg./Kg. of the latter barbiturate, were injected. Tables I and II as well as Fig. 1 show the results of these experiments. The percentage of Nembutal® essentially eliminated per hour is about 28, which equals 11 mg./Kg. Table II shows the essential elimination of Medomin® and reveals the fact that on the average more than 35.7% of the drug is eliminated per hour, which is more than 42 mg./Kg. Nembutal®, therefore is eliminated more slowly than Medomin®, i. e., one-fourth of the fatal dose per hour, as against over one-third of the LD_{50} per hour for Medomin®. In rabbits, Medomin® is definitely a shorter-acting barbiturate than Nembutal®. One should also take into consideration that five rabbits did not die even following the administration of 28 fractional doses, i. e., following injections over a period of fourteen hours, or a total dose of five and one half LD_{50} whereas no animal treated with Nembutal® survived beyond the 26th fractional dose.

COMMENT

In the rabbit, Medomin® is certainly not an intermediate but definitely a short-acting barbiturate,

Following three-fourths of the intravenous LD_{50}, rabbits recovered completely within one hour. One-fifth of the intravenous LD_{50} of either Nembutal® or Medomin® produced little visible depression in the experimental animals, but subsequent doses of Nembutal® produced CNS depression of increasing magnitude, which is also roughly true of the Medomin-treated animals. However, there was one important difference between the Nembutal® and the Medomin® groups. After a few fractional doses, the Nembutalized animals showed no recovery during the time between two fractional doses, whereas the majority of the Medomin-treated animals, although at first depressed, were almost fully recovered before the next injection. In fact, as stated before, some animals recovered even between the 27th and 28th fractional doses.

The more rapid essential elimination of Medomin® could be due to the fact that Medomin® is an unsaturated compound and probably readily metabolized in the tissues (3). It may also be significant that the only oxidation product of Medomin®, cycloheptenylethylbarbituric acid which has been isolated from the urine, produced no anesthetic or hypnotic action whatever (4).

While these results apply only to rabbits, they indicate the general effect that may be expected from the use of these barbiturates. In dogs, and probably also in humans, the duration of action of barbiturates is about twice as long as in rabbits (2). These results, therefore, may not reflect the exact rate of elimination when the drug is administered in therapeutic doses to humans.

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The Physics of Tablet Compression*

VII. Determination of Energy Expenditure in the Tablet Compression Process

By EINO NELSON,† L. W. BUSSE, and TAKERU HIGUCHI

Energy expended in the tablet compression process was calculated by consideration of the magnitude and relation between the several compression variables. In the compression of about 0.4 Gm. of unlubricated sulfathiazole granulation to a maximum force of 1200 Kg. on the lower punch with flat-faced $^3/_8$-inch punches, about 8.6 calories were expended. For about the same weight of lubricated granulation the corresponding value was about 2.0 calories. By considering that only energy expended in the process of eliminating void space to form the tablet caused a temperature rise in the tablet, the temperature rise expected in the lubricated or unlubricated tablets mentioned above would be about 5°. When the energies expended in the processes of tablet ejection, overcoming wall friction and removing the upper punch from the die, were summed for a single punch machine making these tablets at the rate of 100 per minute, it was found that 43 kilocalories would be expended in each hour of operation if the tablets were unlubricated. If lubricated granulation were used the value would be 3 kilocalories. The bulk of these expenditures would probably cause heating of the punches and die and if the granulation was either poorly lubricated or unlubricated the heating might be severe.

THE INSTRUMENTATION of a single punch tablet machine to record the compression variables of upper and lower punch force, ejection force, and upper punch displacement has been recently described (1). The use of this machine to evaluate tablet lubricants by measurement of upper and lower punch forces and tablet ejection force has been recently discussed (2).

In the course of work with this machine it was found possible to calculate the energy expended in the tablet compression process by consideration of the magnitude and relation between the compression variables listed above and the linear dimension of the die cavity. To show the method of calculation the calculations in this paper are for the energy expenditure in compressing about 0.4 Gm. of lubricated and unlubricated sulfathiazole granulation in a $^3/_8$-inch die with flat-faced punches to a maximum compressive force of about 1200 Kg. on the lower punch. The lubricated granulation contained 2% calcium stearate. The total energy of compression was considered that expended from the time the upper punch entered the die until it was drawn free of the die and the finished tablet ejected.

In the compression process contributions to total energy of compression arise from the following processes:

* Received November 3, 1954, from the Research Laboratories, School of Pharmacy, University of Wisconsin, Madison.
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(*a*) Compression of granulation with elimination of void space to form tablet.

(*b*) Overcoming die wall friction in compression.

(*c*) Removal of upper punch from die after compression.

(*d*) Tablet ejection.

CALCULATIONS

The energy expended in compression of granulation to eliminate void space and form the tablet was available from measurement of lower punch force as a function of punch displacement. Since work is defined as

$$W = \int F dx$$

where F is force and x the distance through which it acts, the energy was represented by the area beneath the force-displacement curve. This measurement was based on force at the lower punch since it was more nearly the true force of compression than the force exerted by the upper punch. Upper punch force includes that force needed to overcome die wall friction as well as that used in compression proper. Actually, in any compression where upper and lower punch forces were not the same, the true force of compression was some mean of upper and lower punch forces.

The force-displacement curve for the compression of sulfathiazole granulation is shown as Fig. 1. The area beneath the lower curve is for lower punch force *vs.* upper punch displacement using lubricated granulation. The planimeter measured area beneath this curve is 64,000 gram-centimeters which is equivalent to about 1.5 calories. This is true since one gram-centimeter is equivalent to 2.34×10^{-5} gram-calorie.

The area under the dotted upper curve of Fig. 1 represents energy expended in compression of unlubricated granulation and in overcoming die wall friction since it is a record of upper punch force *vs.* upper punch displacement. The upper curve was obtained from data previously collected in upper and lower punch force studies (2). In these studies it was found that for this granulation, compressed without lubricant added, the upper punch force was about 1.6 times as great as lower punch force. The upper curve of Fig. 1 was made by multiplying each lower punch force value on the lower curve by 1.6 and plotting the result. The area under the upper curve minus that under the lower curve therefore approximately represents energy expended to overcome wall friction. The difference between the two areas was about 34,000 gram-centimeters or about 0.9 calorie.

In the calculation just described the energy value obtained is only approximate since a strictly linear relation did not exist between upper and lower punch force. It was necessary to use the method, however, since upper punch force could not be measured directly as a function of upper punch displacement without introducing uncertainty in punch displacement measurement due to design considerations of the machine.

During the compression of unlubricated sulfathiazole granulation a 50-Kg. force was required to remove the upper punch from the die as can be seen from an examination of Fig. 2.[1] The distance the

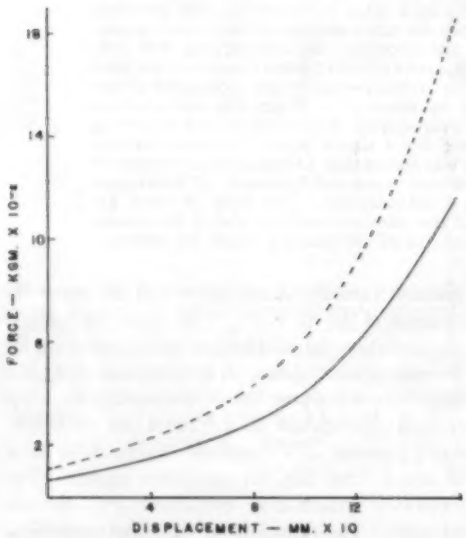


Fig. 1.—Record of upper and lower punch force vs. punch displacement in compression of sulfathiazole granulation. Upper dotted curve is for upper punch force using unlubricated granulation. Lower curve is for lower punch force with lubricated or unlubricated granulation. Lower curve is also for upper punch force for well lubricated granulation.

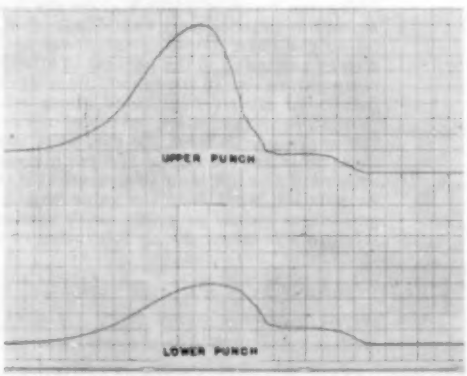
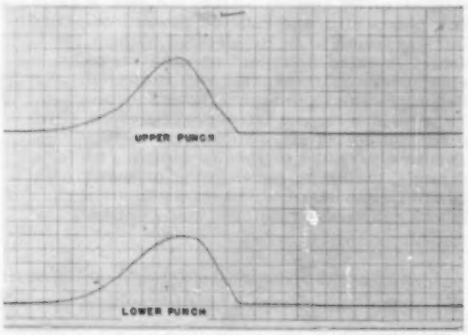


Fig. 2.—Record of upper punch and lower punch force for the compression of unlubricated sulfathiazole granulation. See text.

[1] The two papers previously published did not discuss the fact that the force necessary to remove the upper punch from the die was available from the oscillograph record for the upper punch. An examination of Figs. 2 and 3, respectively, for the compression of unlubricated and lubricated granulation, will show that in the case of the unlubricated granulation the upper punch force curve drops about 50 Kg. below the baseline used for measuring upper punch force before it drops still farther when tablet ejection begins. It will be evident that this initial drop below the baseline corresponds to the force necessary to remove the upper punch from the die by examination of the curve for the lubricated granulation where the curve falls just to the baseline and then dips slightly during tablet ejection.

upper punch moved while overcoming this force was about one centimeter. Energy expended for this process was then 50,000 gram-centimeters or about 1.2 calories. The force necessary to remove the upper punch from the die when the granulation was lubricated was negligible as can be seen from examination of Fig. 3. Consequently, no significant amount of energy was expended in the process.



EACH CHART DIVISION = 1 mm. = 40 Kg. FORCE

Fig. 3.—Record of upper and lower punch force for compression of sulfathiazole granulation lubricated with 2 per cent calcium stearate.

The force necessary for ejection of the tablet made from unlubricated granulation was 220 Kg. The ejection distance was about one centimeter so the energy expended in the process was 220,000 gram-centimeters or about 5.1 calories. When lubricated granulation was used the ejection force was about 20 Kg. The energy expended then was 20,000 gram-centimeter or about 0.5 calorie.

Ejection forces for the determination just described were read from the oscillograph records for the compression of unlubricated and lubricated granulation, respectively, shown as Figs. 2 and 3, as previously described (1, 2).

The energy values obtained in all the preceding calculations are listed and totaled on Table I both for the lubricated and unlubricated granulation.

The temperature rise expected in a tablet may be calculated from the thermal capacity of tablet material and energy expenditure in the process under consideration. By assuming that all of the energy expended appeared as heat, the temperature rise was calculated by the following:

$$\Delta T = Q/CM$$

where ΔT is the rise in temperature in degrees centigrade, Q the heat in calories input, C the thermal capacity of the granulation, and M the weight in grams of granulation compressed. By substitution, the temperature rise expected from energy expended just in compression to eliminate void space from 0.4 Gm. of either lubricated or unlubricated granulation was found to be about 5°. The thermal capacity of sulfathiazole granulation was taken to be 0.75 calorie/degree/gram.[3] The total heat effect would be higher with unlubricated granulation if some of the energy expended for processes other than compression to eliminate void space appeared as heat in the tablet.

[3] The thermal capacity of an organic compound can be found approximately by taking the reciprocal of its density.

DISCUSSION

It is immediately apparent from an examination of Table I that the bulk of total expenditure arose from tablet ejection when unlubricated granulation was compressed. It is also apparent that an efficient lubricant caused a large change both in this quantity and total expenditure required.

It is of interest to consider the disposition of energy expended in each of the four processes that contributed to total energy expenditure. In compression to eliminate void space and form the tablet, the First Law of Thermodynamics requires that energy expended manifest itself as heat or change in internal energy of the compressed granulation. The bulk of the energy expended in this process probably manifested itself by increasing the temperature of the tablet and this was assumed when the temperature rise calculation was made.

In the processes of overcoming wall friction, removing the upper punch from the die, and in tablet ejection the bulk of the energy expended probably appeared as heat in the punches and die due to the relatively high coefficient of thermal conductivity of these materials as compared to the conductivity coefficients of most materials made into tablets.

Depending on opportunities for heat diffusion, appreciable local heating may be possible in compression of unlubricated or poorly lubricated granulation. In a single punch tablet machine making 100 tablets per minute of sulfathiazole, at the compressive force and of the unlubricated composition made here, about 43 Kcal. of energy would be available to heat the punches and die and associated machine parts in every hour of operation. With well-lubricated granulation the energy available would be only 3 Kcal. during the same period. These energy values do not include energy expended in compression to eliminate void space to form tablets.

TABLE I.—ENERGY EXPENDED IN COMPRESSION OF 0.4 GM. OF SULFATHIAZOLE GRANULATION IN A SINGLE PUNCH TABLET MACHINE[a]

	Compression Process	Energy Expended (Calories) Unlubricated	Lubricated
1.	Compression	1.5	1.5
2.	Overcoming wall friction	0.8	[b]
3.	Upper punch withdrawal	1.2	[b]
4.	Tablet ejection	5.1	0.5
	Total	8.6	2.0

[a] To a maximum force of about 1200 Kg. on the lower punch using ⅝-inch flat-faced punches.
[b] Negligible.

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The Decomposition of Aspirin in Aspirin, Phenacetin, and Caffeine Tablets*

By DINIZ RIBEIRO, DALE STEVENSON, JOSEPH SAMYN, GEORGE MILOSOVICH, and ALBERT M. MATTOCKS

Through tests on tablets made in our own laboratories and on market samples, it was noted that many formulations of aspirin, phenacetin, and caffeine showed decomposition of aspirin. The decomposition was found to be accelerated by heat. Discussion with experienced tablet formulators revealed divergent opinions as to causes of aspirin decomposition. A series of preliminary experiments indicated that type of lubricant was the main cause of decomposition, although it seemed inconceivable that moisture, pressure, and type of aspirin were not factors. Therefore, a factorial experiment was set up testing all combinations of lubricants at two levels, pressure at two levels, moisture at two levels, and two types of aspirin (10 per cent starch granulation and 40-mesh crystal), the entire series being repeated for each of seven different lubricants. A total of 112 batches of tablets were prepared. The tablets were aged at 45° for four weeks and analyzed for salicylic acid content.

IN THE COURSE of setting up a formula for aspirin, phenacetin, and caffeine tablets, it was found that the majority of the formulas tested were unstable. The instability was apparently due to aspirin decomposition with the release of acetic acid which could be detected easily by odor. Storing the tablets at 45° accelerated the decomposition rate, and this storage condition was used to test all of the formulas. Similar tests on a number of commerical tablets showed several of these products to decompose in the same manner.

It has been noted in the literature that aspirin when combined with phenacetin and caffeine or codeine has often been found to be unstable, and a number of formulas have been suggested (1–5).

A series of preliminary experiments in this laboratory gave indications that certain lubricants caused more rapid decomposition of aspirin than others, and it was thought that the use of high pressure in compression of the tablets was a cause of decomposition. In discussions with industrial laboratories, it was frequently stated that high moisture content of the tablets was the chief cause of aspirin decomposition, and this seems to be a logical explanation since moisture is required for the hydrolysis reaction. On the other hand, some laboratories maintain a rather high moisture content in the tablet mixture in order to improve compression characteristics. Others have expressed the opinion that decomposition occurs with granular type aspirin and not with the crystalline variety.

Owing to the divergence of opinion on the cause of aspirin breakdown in this formula, it was felt that whatever experiments were conducted should take into consideration these different possibilities. It was conceivable that any one or any combination of these factors might be responsible. The complexity of the problem along with the interest of this laboratory to develop and test more direct and useful approaches to stability problems led the authors to set up a factorial experiment in this study. The advantage of the factorial experiment is that it makes possible the evaluation of effects of all combinations of factors, thus giving the maximum amount of information in a relatively short time. This experiment is estimated to have required about eighty man-hours of laboratory work and sixteen hours of calculations on a semi-automatic machine.

EXPERIMENTAL

A $2 \times 2 \times 2 \times 2 \times 7$ factorial experiment was set up, testing moisture, lubricant, and pressure, each at two levels, granular and crystalline aspirin, and seven different kinds of lubricant. Tablet granulations were made by the following formula:

Phenacetin-caffeine granulation (phenacetin 16.0, caffeine anhydrous 3.2, granulated with starch-sugar paste)	19.2
Aspirin	22.7
Lubricant, fixed amounts (see under Materials Used)	
Starch, *q. s. ad*	46.4

When granular aspirin was used, the formula was adjusted to allow for the starch already present.

Materials Used.—Phenacetin-caffeine granulation, high moisture, prepared by above formula and dried at room temperature eighteen hours. Moisture content 1.64%.

Phenacetin-caffeine granulation, low moisture, prepared as above and dried at 120°F. seventeen hours. Moisture content 0.098%.

* Received August 27, 1954, from the Manufacturing Pharmacy Laboratories, College of Pharmacy, University of Michigan.

Presented to the Scientific Section, A. PH. A., Boston meeting, August, 1954.

The authors wish to express their gratitude to Professor Paul S. Dwyer, Department of Mathematics, University of Michigan, for his advice and aid in setting up the methods of statistical analysis, and to D. M. Patel, John Sciarra, M. S. Patel, Werner Lowenthal, and P. R. Sheth, graduate students in the College of Pharmacy, for their assistance with the chemical analyses.

TABLE I

Combination*	PER CENT ASPIRIN DECOMPOSED						
	Aldo 33®	Magnesium Stearate	Talc	Calcium Stearate	Stearic Acid	Mineral Oil	Talc Plus Mineral Oil
MPAL	1.17	8.67	1.10	20.95	8.03	4.11	2.97
MpAL	1.76	8.31	0.97	19.65	8.54	4.59	1.33
MPaL	0.67	7.06	0.55	21.21	0.98	0.99	0.30
Mpal	0.36	2.04	0.00	6.39	0.69	0.39	0.52
MPal	0.33	1.21	0.00	6.95	0.84	0.31	0.22
MpAl	1.63	3.20	2.76	6.20	3.48	4.70	0.87
MpaL	1.05	6.26	0.00	19.54	2.00	0.99	0.75
MPAl	0.99	2.28	2.21	3.84	1.27	2.40	1.16
mPAL	8.56	6.84	1.41	16.04	7.67	1.18	2.66
mpAL	8.59	7.71	2.65	18.35	12.51	2.03	0.58
mPaL	0.96	15.99	0.54	18.54	3.95	0.92	0.87
mpal	0.80	3.39	0.55	3.53	0.44	0.45	0.97
mPal	0.53	3.03	0.48	4.45	1.28	0.90	0.70
mpAl	0.82	2.45	0.49	8.09	2.09	0.68	0.63
mpaL	0.45	15.26	0.73	19.79	4.14	0.93	0.98
mPAl	1.00	3.22	0.73	6.31	3.84	1.06	0.16

* M—moisture, P—pressure, L—lubricant, capital letters denoting high level, lower case denoting low levels. A—granular aspirin, a—crystalline aspirin.

Aspirin granules, commercial 10% starch granulation, 14-mesh, made by direct compression.

Aspirin crystals, commercial 40-mesh crystals.

Lubricants—all pharmaceutical grade in the following quantities:

Lubricant	High Level, %	Low Level, %
Aldo 33®[1]	2	0.5
Magnesium stearate	2	0.5
Talc	4	1.0
Calcium stearate	2	0.5
Stearic acid	2	0.5
Light mineral oil	4	1.0
Talc plus light mineral oil, equal parts	4	1.0

Procedure.—The mixtures of tablet ingredients were prepared by tumbling in wide-mouth bottles. All 16 combinations of four factors at two levels for seven lubricants were prepared (Table I). The two levels of pressure were obtained by pressing one set of mixtures at as low a pressure as possible to obtain a tablet, and the second set at the maximum pressure of the machine.

Throughout every step of preparation and analysis, the mixtures were handled in random order.

The tablets, in loosely capped bottles, were placed in an incubator at 45° for twenty-seven days. At the end of this time they were analyzed for salicylic acid content by a modification of the A. O. A. C. method (6).

Analysis.—Fifteen tablets were ground thoroughly in a mortar, and samples of approximately 0.2 Gm., accurately weighed, were placed in 125-ml. Erlenmeyer flasks. Twenty-five milliliters of absolute ethanol was added, the mixture was shaken mechanically for thirty minutes and filtered. A 10-ml. aliquot of the filtrate was transferred to a 100-ml. volumetric flask. Twenty-five milliliters absolute ethanol, 2 ml. ferric ammonium sulfate test solution (freshly prepared by diluting 25 ml. 8% ferric ammonium sulfate reagent solution with 12.5 ml. normal hydrochloric acid and water to make 100 ml.) were added, and the solution was made up to volume with water. Solutions were read against a reagent blank at 537 mμ on a Beckman DU Spectrophotometer. Concentrations were obtained from a standard curve of salicylic acid. Samples of freshly compressed tablets of stable composition and of crystalline aspirin were found to give zero readings by this method.

Results.—The results, obtained in terms of salicylic acid formed, were all converted to per cent of aspirin content which had decomposed. Table I shows the results obtained with the various mixtures.

Table II shows the analysis of variance of the data. Note that the factors showing significant effect unassociated with higher order interaction are: B (types of lubricant), LB (type lubricant-level of lubricant interaction), AB (type lubricant-type aspirin interaction), MAB (type lubricant-type aspirin-moisture level interaction), ALB (type lubricant-level of lubricant-type of aspirin interaction), L (level of lubricant), MALB (type lubricant-type aspirin-moisture level-level of lubricant interaction).

Tables III, IV, and V show the mean values for decomposition of aspirin in those samples depicting the significant effects. This type of arrangement of data makes it a bit easier to see at a glance the effects of single factors and combinations of factors on the decomposition of aspirin in the tablets.

DISCUSSION AND CONCLUSIONS

The results show large and significant differences in decomposition rates due to type of lubricant (large B effect). From this experiment it seems that lubricants in order of increasing effect are talc, talc plus mineral oil, mineral oil, Aldo 33®, stearic acid, magnesium stearate, calcium stearate. The first four of these are not significantly different from one another but as a group show considerably less decomposition than the other three. The high level of lubricant caused a general increase in decomposition (L effect) and a striking increase when the lubricant type was one causing a large effect, such as calcium stearate or stearic acid (LB effect).

[1] Glycerin monostearate (Glyco Products Co., Inc.).

TABLE II.—ANALYSIS OF VARIANCE

Source[a]	No. Groups	ΣX^2	L[c]	L or I	d.f.		F vs. 5-Factor[b] Interaction	F vs. Interactions Involved
M	2	99564.2141	488.8521	488.8521	1	488.8521	9.50*	
A	2	101727.2741	4814.9721	4814.9721	1	4814.9721	93.6***	
L	2	124648.0405	50656.5049	50656.5049	1	50656.5049	984.8***	7.19*
P	2	99398.0381	156.5001	156.5001	1	156.5001	3.04	
B	7	55209.8989	187829.7162	187829.7162	6	31304.95	608.***	9.79***
MA	4	51127.3411	5869.7883	565.9641	1	565.9641	11.0*	
ML	4	62678.0339	52072.5595	927.2025	1	927.2025	18.0**	
MP	4	49821.2321	645.3523	0.0001	1	0.0001	<1	
MB	14	28027.6167	193747.0577	5428.4894	6	904.7482	17.58*	
AL	4	63589.9365	55720.1699	248.6929	1	248.6929	4.83	
AP	4	50960.9027	5204.0347	232.5625	1	232.5625	4.52	
AB	14	28577.7781	201449.3173	8804.6290	6	1467.4381	28.5***	3.71**
LP	4	62363.5743	50814.7211	1.7161	1	1.7161	<1	
LB	14	35740.2595	301724.0569	63237.8358	6	10539.639	204.***	25.75***
PB	14	27637.2649	188282.1325	295.9162	6	49.3194	<1	
MAL	8	32056.3709	57811.3911	109.2025	1	109.2025	2.12	
MAP	8	25612.3079	6258.8871	0.0361	1	0.0361	<1	
MAB	28	14727.5777	213732.5995	5799.9766	6	966.6677	18.79**	3.62*
ALP	8	31844.0641	56112.9367	1.9881	1	1.9881	<1	
APB	28	14335.3231	202749.4707	615.1746	6	102.5291	1.99	
LPB	28	17905.3815	302711.1059	532.2249	6	88.8194	1.73	
MLP	8	31425.3221	52763.0007	532.2249	1	532.2249	10.34*	
MLB	28	18203.2551	311051.5667	2482.9658	6	413.8276	8.04*	
MBP	28	14041.8391	194531.9187	332.4446	6	55.40743	1.08	
ALB	28	18563.8203	321147.3923	5555.0414	6	925.8402	17.99**	3.47*
MALP	16	16102.3415	58997.8879	261.4689	1	261.4689	5.08	
MALB	56	9660.8651	342368.8695	5418.8242	6	903.1373	17.55**	17.55**
MLPB	56	9144.8545	313472.2759	568.9906	6	94.83176	1.843	
ALPB	56	9321.2347	32349.5671	365.4006	6	60.90010	1.183	
MAPB	56	7393.9269	215420.3303	55.0966	6	9.18276	<1	
			Subtotal	346,321.3061				
MALPB				308.6202	6	51.4367		
Total	112			346,629.9263	111			

[a] Letters have following meanings: M, m—high and low moisture, resp.; A, a—granular and crystalline aspirin; L, l—high and low level of lubricant, resp.; P, p—high and low pressures, resp.; B—kinds of lubricant. (See Tables IV and V.)
[b] * denotes significance at 5% level; **, at 1%; ***, at 0.1% level.
[c] Notations as developed by Paul S. Dwyer ("Linear Computations," John Wiley & Sons, Inc., New York, 1951, p. 302).

TABLE III.—TYPES OF LUBRICANT AND LEVELS OF LUBRICANT

Lubricant	Overall Mean Decompn., %	Mean Decompn., % High	Low
Aldo 33®	1.85	2.90	0.81
Magnesium stearate	6.06	9.51	2.60
Talc	0.95	0.99	0.90
Calcium stearate	12.49	19.25	5.68
Stearic acid	3.86	5.98	1.74
Mineral oil	1.66	1.97	1.36
Talc plus mineral oil	0.98	1.31	0.65

plus mineral oil, the crystalline aspirin having superior stability in most cases (AB and ALB effects). Moisture content also influenced this effect, acting in a surprising manner in several instances, high moisture causing less decomposition (MAB effect). Pressure levels showed no effect in the experiment.

It may be concluded from these results that the aspirin decomposition in tablets of APC may be minimized by using talc, mineral oil,

TABLE IV.—TYPE OF ASPIRIN, TYPE OF LUBRICANT, AND LEVEL OF LUBRICANT

Lubricant	Granular Aspirin: Mean L + l	Mean L	Mean l	Crystalline Aspirin: Mean L + l	Mean L	Mean l
Aldo 33®	3.07	5.02	1.11	0.64	0.78	0.51
Magnesium stearate	5.34	7.88	2.79	6.78	11.14	2.42
Talc	1.54	1.53	1.55	0.36	0.46	0.26
Calcium stearate	12.43	18.75	6.11	12.55	19.77	5.33
Stearic acid	5.94	9.20	2.67	1.79	2.77	0.81
Mineral oil	2.59	2.98	2.21	0.74	0.96	0.51
Talc plus mineral oil	1.30	1.89	0.71	0.66	0.73	0.60

Type of aspirin was found to be important when certain lubricants were used, particularly with Aldo 33®, stearic acid, mineral oil, and talc plus mineral oil, or Aldo 33® as the lubricant, in combination with crystalline aspirin. Further, moderate moisture content of the

TABLE V.—MOISTURE LEVEL, TYPE ASPIRIN, TYPE LUBRICANT

Lubricant	Granular Aspirin			Crystalline Aspirin		
	Mean M + m	Mean M	Mean m	Mean M + m	Mean M	Mean m
Aldo 33®	3.07	1.39	4.74	0.64	0.63	0.69
Magnesium stearate	5.34	5.62	5.06	6.78	4.19	9.42
Talc	1.54	1.76	1.32	0.36	0.14	0.58
Calcium stearate	12.43	12.66	12.20	12.55	13.52	11.58
Stearic acid	5.94	5.34	6.53	1.79	1.13	2.45
Mineral oil	2.59	3.95	1.24	0.74	0.67	0.80
Talc plus mineral oil	1.30	1.58	1.01	0.66	0.45	0.88

phenacetin-caffeine granulation does not increase decomposition, and the pressure of the ordinary tablet machine is not sufficient to cause decomposition.

As a result of this study the following formula for APC tablets was developed and found to be relatively stable and quite acceptable as to hardness, appearance, and disintegration:

Acetophenetidin	0.160 Gm.
Caffeine anhydrous	0.032 Gm.
(Granulate with 10% starch paste in equal parts water and syrup)	
Acetylsalicylic acid, 40-mesh	0.227 Gm.
Starch	0.042 Gm.
Talc	0.008 Gm.

This work illustrates the advantages of investigating complex stability problems by factorial experiments, especially when two or more factors are suspected. At the same time it offers another example of the unexpected type of reaction occurring in tablet compositions.

Further experiments are planned to learn more of the nature of the reactions occurring in the APC formula.

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Apparatus and Technique Involved in a Laboratory Method of Detecting the Addictiveness of Drugs*

By CECIL P. HEADLEE, HAROLD W. COPPOCK, and JOHN R. NICHOLS

A new procedure for the laboratory determination of drug addiction has been devised and subjected to a preliminary trial using rats as experimental subjects. Automatic equipment has been constructed and utilized to produce permanent records indicating the influence of morphine, codeine, glucose, saline, and insulin administration to pretreated and deprived animals. Preliminary data indicate the method to be worthy of further study.

MANY CURRENT STUDIES of drugs showing an addiction liability have been directed toward histological, biochemical, or neurological changes in animals following the administration of the drug in question. The results of such studies, though of vital importance, have not yet presented a technique which will provide a reliable index of addictiveness of new drugs. The one index of addictiveness now in common use is the behavior of the patient administered the drug. Spragg (1) and Himmelsbach (2) utilized this index as a basis of testing drugs for addiction properties. To date, however, the authors are aware of no other such research and, more important, are aware of no acceptable method of detecting the addictiveness of new drugs. In view of this, an attempt has been made to devise a procedure to indicate such properties more clearly and precisely.

APPARATUS

The automatic equipment, partially described elsewhere (3), consisted of one unit in which all rats were tested successively. The outer compartment was an insulated aluminum box, maintained at a

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temperature of 28 to 32° through the use of a small ventilating fan. Inside and clamped to a transparent floor, 2 inches above the bottom of the box, was a quonset-shaped metal cage, designed so that adjustments could be made to hold rats of varying sizes. The cage, used to restrain the rats throughout the test, was designed with a slot in the front, 1¾ inches high and ¾ inch wide, so as to fit snugly around the neck of the animal. Provisions were made so that a metal bar could be inserted across the bottom of the slot, to press firmly against the chest of the rat. The rear of the cage was equipped with a small V-shaped slot through which the tail of the rat protruded. The tail was taped to a 5-inch horizontal bar welded to the cage above the slot. Thus, with the cage placed around the rat, with the metal chest bar in place, and with the tail tightly taped, both forward and backward movement of the animal was prevented and the activity of the animal was narrowed to occasional postural adjustments and frequent head movements.

Under the transparent plastic floor and to each side of the rat's head (when positioned in the restraining cage) were placed two clear, 7-watt 115-volt light bulbs from which most of the heat and visible light had been filtered by a thin bottle of a saturated solution of iodine in 50% alcohol. These light sources were to provide vertical beams of infrared light to photoelectric cells which were actuated by interruption of the light beams resulting from lateral head movements of the animal restrained in the cage.

Adjustments were made until a rotation of a rat's head to a position more than 30° off center actuated the relay on that side. Once actuated, this relay remained closed until the head of the animal was moved back to less than 20° off center. Each relay was connected to and controlled an ink pen of a polygraph recorder. The paper chart of this recorder was set to move at 1.5 inches per minute.

To provide liquid reinforcement to the animals while restrained in the cage and inside the soundproof box, an automatic injection apparatus was devised. This apparatus consisted of a screw principle actuator driven by a one r. p. m. electric motor. This design was such that rotation of a screw principle actuator would compress the plunger of a hypodermic syringe secured in a stationary holder. A flexible plastic tubing and a 26-gauge, ¾-inch needle were connected to the syringe to convey the liquid reinforcement into the peritoneal cavity of the rat. The electric motor operating the syringe could either be connected to the photoelectric relays or entirely disconnected from the circuit. Further, the motor was connected to a pen of the polygraph recorder so that permanent records could be made of the operation of the injection motor. The rate of liquid injection was set at 0.1 cc. per minute. All this apparatus was fashioned so that the relays, motors, recording pens, and other noise-producing devices were located outside the soundproof box.

EXPERIMENTAL

Morphine, Codeine, and Control Animals.—A group of 30 male rats of the Sprague-Dawley strain weighing between 150 and 240 Gm. was selected for the experiment. Animals from this group were randomly assigned to one of three treatment groups

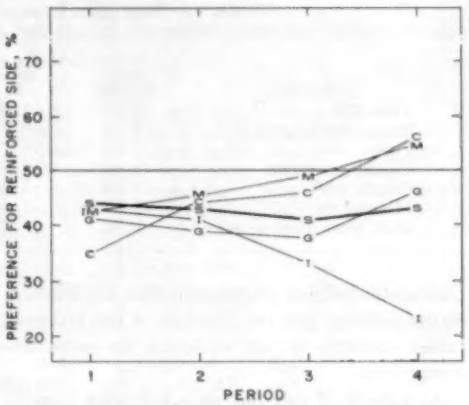

Fig. 1.—Change in per cent preference following reinforcement of originally nonpreferred side with indicated drug. ——M——morphine, ——C—— codeine, ——S—— saline, ——G—— glucose, ——I—— insulin.

until a total of eight rats were assigned to each group. All groups received injections of the same relative volume, that is, 4 cc./Kg., each day for a period of two weeks. Group 1 received daily intraperitoneal injections of 20 mg./Kg. of morphine hydrochloride in normal saline solution, Group 2 were administered daily intraperitoneal injections of 60 mg./Kg. of codeine sulfate in normal saline solution and Group 3, the control group, received daily intraperitoneal injections of 4 cc./Kg. of normal saline solution. The tranquilizing doses (4) of morphine and codeine were considered to be sufficient to produce addiction in the rats by the end of the two-week period of injections. After having received 14 daily injections, the animal received no further injections for forty-eight hours. The resulting hyperirritability upon handling the opiate-treated animals, as well as restlessness and introversion when left undisturbed in the cage, was taken as evidence of withdrawal symptoms and addiction. Following this forty-eight-hour interval of time, the rat was positioned in the restraining cage and the hypodermic needle was inserted into the peritoneal cavity and secured in place. The cage and rat with the needle in position were then clamped into place in the aluminum box and the polygraph recorder was started. The automatic injection device, however, was not immediately connected to the photoelectric relays so that the rats received no liquid reinforcements for any head movements. After a ten-minute period, the polygram was then examined and if it was observed that the rat had held its head longer on the right than on the left, then the injection device was connected to the left photoelectric relay, i. e., the injection device was set to provide a liquid reinforcement to the rat when the animal moved its head to the side on which it had spent the least amount of time during this first ten-minute period (the nonpreferred side). After turning on the liquid reinforcements, i. e., following the first ten-minute period, the rat was then left in the testing apparatus for a period of ninety minutes. Following this, and as no cross tests were attempted, the rat was removed from the box and from the cage to conclude the testing of that animal. For pur-

poses of analysis and drawing of figures, the test was divided into four periods, i. e., a ten-minute adaptation period and three test periods of thirty minutes. All animals in all three groups were tested in this manner.

As may be seen in Fig. 1, the net change scores in per cent preference between Periods 1 and 4 gave mean differences of plus 12% for the morphine group, plus 20% for the codeine group, and 0% for the saline group. A *t*-test for related measures made on difference scores revealed the morphine group to be significant at the 0.05 level of confidence and the codeine group significant at the 0.001 level. There was no statistical significance between Periods 1 and 4 in the saline group.

Insulin- and Glucose-Treated Animals.—If the results of the morphine-codeine study are due to the restoring of homeostatic equilibrium, then the administration of glucose to hungry animals should be qualitatively the same as administration of morphine or codeine to the addicted animals. Further, administration of insulin to hungry animals should be qualitatively opposite. In an attempt to show these postulations to be true, another study has been made. Although the basic idea remained essentially the same, of necessity, the procedure involved differed somewhat from that of the morphine-codeine study. Forty Sprague-Dawley male rats weighing from 150 to 250 Gm. were separated into two groups. One group of 18 animals was utilized in the insulin study and the remaining 22 in the glucose study. After being fasted for seventy-two hours the rats were positioned in the restraining cage and the hypodermic needle was inserted intraperitoneally and secured in place. The cage was then clamped into place in the automatic conditioning box. After ten minutes of adaptation, i. e., to determine the nonpreferred side, the animal was administered either 20% glucose in normal saline or 1.82 units per cc. of insulin in normal saline. The automatic injection device was set to pump the liquid reinforcement into the rat at a rate of 0.1 cc per minute as long as the rat's head was on the nonpreferred side. As in the previous study, the animal was left in the cage for a ninety-minute test period, and for purposes of analysis and drawing of figures the test was divided into four periods, a ten-minute period and three periods of thirty minutes each.

When the net change scores in per cent preference between Periods 1 and 4 were determined for these animals, the glucose group showed a mean difference of plus 5% while the insulin group showed a mean difference of minus 19%. Although there could be demonstrated no statistical significance between Periods 1 and 4 in glucose group, it is interesting to note the trend in the direction predicted by the hypothesis. A *t*-test showed the insulin group to be significant at the 0.06 level of confidence.

SUMMARY

1. A method for the laboratory determination of drug addiction is presented. The method is based on the appeasement of withdrawal symtoms and restoration of homeostatic equilibrium.

2. The method has been subjected to a preliminary investigation utilizing morphine, codeine, glucose, and insulin as test drugs. The resultant preliminary data have been considered sufficient to warrant further study utilizing the apparatus and procedure described.

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Interference of Sucrose in the Plate Assay of Polymyxin B*

By N. KNOWLDEN, E. BROOMFIELD, and J. HERNAN

A statistical study of the interference of sucrose in the polymyxin B plate assay is described. Ninety-five per cent of the existing variation is explained. The study enables the nature of the interference to be determined and presents a means of correction when estimating polymyxin B potencies.

IN THE COURSE of assaying polymyxin B products by a plate assay technique, it appeared that the presence of sucrose in a sample could interfere in the assay of polymyxin B. An investigation was therefore pursued to determine the nature of this effect and how it could be compensated in the assay of polymyxin B.[1]

EXPERIMENTAL

Materials and Methods.—The assay used the organism *Brucella bronchiseptica ATCC 4617* and the procedure of Knowlden (1). Since the dilutions normally assayed contained a combination of 100 units of polymyxin B and either 10 or 20 mg. of sucrose per ml., solutions for this study were prepared, in glycine buffer *p*H 2.0, to investigate this area. From previous work it was known that solutions of sucrose alone in these concentrations had no measurable effect on the organism under these

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The authors wish to express their gratitude to Dr. C. W. Ferry, Chief Control Chemist, for his interest in this study, and to the members of the antibiotic assay staff who aided in performing the assays necessary.
[1] Polymyxin B is marketed under the trade name of "Aerosporin"®.

conditions. The solutions prepared and the results obtained from assay are presented in Table I where each figure represents a zone diameter average for 15 plates.

Results.—An analysis of variance (2) performed on the data in Table I showed that the sucrose had a statistically significant effect as well as the polymyxin B and that there was no significant interaction between the two. Assuming that the polymyxin B effect could be made linear by using the ordinary logarithmic transformation (3) the problem remained as to the linearity of the sucrose effect. The sucrose effect was plotted on linear graph paper, a separate line drawn for each polymyxin B concentration. The overall appearance was a family of straight parallel lines with a few aberrations.

TABLE I.—AVERAGE DIAMETER OF INHIBITION ZONE IN MM. FOR POLYMYXIN B—SUCROSE COMBINATIONS

Sucrose, mg./ml.	Polymyxin B (units/ml.) 60	80	100	120	140
0	13.67	14.28	14.86	15.25	15.46
	13.86	14.48	14.82	15.33	15.61
2.5	14.17	14.56	14.93	15.26	15.44
	14.15	14.38	14.86	15.28	15.40
5.0	13.78	14.36	14.85	15.15	15.56
	14.11	14.40	14.83	15.25	15.63
10.0	13.83	14.45	14.93	15.15	15.49
	14.16	14.44	14.74	15.34	15.57
20.0	13.70	14.40	15.28	15.48	15.78
	14.52	14.89	14.94	15.71	16.15
40.0	14.49	15.13	15.44	15.99	16.30
	14.99	15.21	15.60	16.04	16.35
60.0	14.61	15.26	15.84	16.18	16.51
	14.74	15.46	15.95	16.24	16.44

With this information a multiple regression analysis (4) was conducted on the data. This yielded the equation:

$$y = 5.8160 + 4.5038X_1 + 0.0174X_2$$

where y is the inhibition zone diameter, X_1 is the common logarithm of the units of polymyxin B present per ml. and X_2 is the mg. of sucrose present per ml. This regression equation expresses the result when the antibiotic and sucrose are present at varying levels. A breakdown of the analysis of variance (Table II) depicts the improvement effected by using the multiple regression technique.

From the parameters of the multiple regression equation (Table III) we see that there is no interaction between the two effects, cov $(b_1b_2) = 0$, although each ingredient does affect the intercept, i. e., cov (ab_1) and cov (ab_2) both exist. Consequently the sucrose effect is of a linear form and may be compensated.

Comparing the polymyxin regression coefficient to that found for the composite curve (1) by a t test (2), it was found there was no significant difference. The composite curve or the regular standard curve (5) can therefore be used to estimate polymyxin potencies when sucrose is present provided a correction is made for the sucrose effect. This may be done by including in the reference dilution an amount of sucrose equivalent to that in the dilution under assay. In this manner correction is made for the complete linear effect of the sucrose, i. e., both the constant effect on the intercept due to sucrose being present and the varying effect due to the concentration of that sucrose present.

When several products containing differing amounts of sucrose are under assay, this procedure becomes cumbersome since references must be prepared for each product. To alleviate this condition a nomograph (Fig. 1) was constructed to be used as follows:

The dilution to be assayed for polymyxin B is prepared (1) and the concentration of sucrose present per ml. in this dilution is determined. Include on each assay plate a reference solution prepared to contain a known concentration of both the antibiotic and the sucrose. The difference between the average zone diameter for this reference and that value on the nomograph corresponding to these known concentrations is used to adjust the other average zone diameters to correct for plate differ-

TABLE II.—ANALYSIS OF VARIANCE OF POLYMYXIN B—SUCROSE DATA WITH PERCENTAGE OF TOTAL VARIATION EXPLAINED BY EACH SOURCE OF VARIATION

Variation Explained by	d/f	Sum of Squares	Mean Square	F' [a]	F'' [b]	Total Variation, %	
Polymyxin B	4	24.0413	6.0103	206.5[c]	...	68.8	..
Regression	*1*	*23.9845*	*23.9845*	...	*894.9*[c]	...	*68.6*
Deviations	*3*	*0.0568*	*0.0189*	0.6	...	...	*0.2*
Sucrose	6	9.5128	1.5854	54.5[c]	...	27.2	..
Regression	*1*	*9.1751*	*9.1751*	...	*342.4*[c]	...	*26.2*
Deviations	*5*	*0.3377*	*0.0675*	2.3	...	...	*1.0*
Experiment Residual	24	0.3830	0.0159	0.5	...	1.1	..
Experiment	34	33.9371	...	...	...	97.1	..
Replicates	35	1.0211	0.0291	...	...	2.9	..
Total	69	34.9582	...	...	...	100.0	..
Multiple Regression	*2*	*33.1596*	*16.5798*	...	*618.6*[c]	...	*94.9*
Deviations (Error)	*67*	*1.7986*	*0.0268*	...	...	...	*5.1*

[a] F' = Mean Square/Mean Square for Replicates; [b] F'' = Mean Square/Mean Square for Error; [c] significant at $P = 0.001$

TABLE III.—PARAMETERS OF MULTIPLE REGRESSION ANALYSIS OF TABLE I DATA

Intercept	Regression Coefficients		Error Variance	Regression Coefficient Variances		Intercept Variance	Covariances	
a	b_1	b_2	σ_e^2	σ_{b1}^2	σ_{b2}^2	σ_a^2	Cov(ab_1)	Cov(ab_2)
5.8160	4.5038	0.0174	0.0268	0.0453	0.000002	0.1794	−0.0898	−0.00003
							Cov(b_1b_2) = 0	

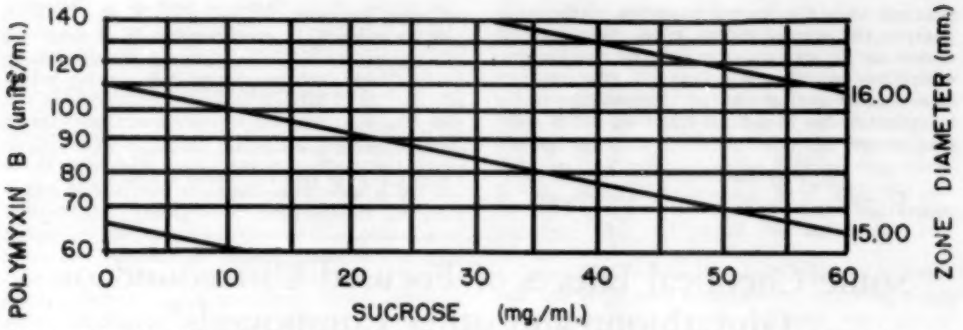

Fig. 1.—Nomograph for determining units of polymyxin B present per ml. when concentration of sucrose present is known.

ences before applying the nomograph. The result of the polymyxin B assay must fall somewhere on the vertical line expressing the sucrose concentration present in the sample under assay. From the polymyxin B assay obtain the adjusted average diameter of the zone of inhibition which may be expressed by a diagonal line on the nomograph. Where these two lines intersect, ascertain the polymyxin B concentration in units per ml. which is the horizontal line passing through that point.

As an example consider estimating the potency of a troche with a labeled claim of 5000 units polymyxin B which contains 950 mg. of sucrose. The dilution to be assayed is prepared to contain 100 units polymyxin B per ml. Also present is 19 mg. sucrose per ml. This assay dilution is applied to the plates along with a reference dilution containing 100 units polymyxin B and 20 mg. sucrose per ml.; the other four cylinders are used for other samples. Results from an actual assay are presented in Table IV. The average reference zone diameter found, 15.50 mm., differs from the nomograph value representing this reference combination, 15.17 mm., by 0.33 mm. This difference, due primarily to plate differences, is used to adjust the sample averages on that set of plates. The resulting corrected sample average, 15.30 mm., with the 19 mg. sucrose per ml. known to be present represents a point on the nomograph. The horizontal line through that point, 106 units per ml., expresses the polymyxin B content of the dilution under assay. Consequently the troche potency may be estimated as 106 per cent labeled claim or as 5300 units polymyxin B per troche.

For practical usage it is advisable to construct the nomograph on two-cycle semilogarithmic paper (K and E 359-61) by calculating several points from the multiple regression equation. This reduces the interpolation necessary on both the horizontal and vertical axes. Interpolation on the diagonal axis may be reduced by constructing parallel lines to represent intermediate zone diameters.

In essence this is equivalent to applying the composite curve correction but enables assays to be conducted on dilutions containing differing sucrose concentrations with only one reference dilution necessary.

Since polymyxin B is an independent variable in the multiple regression analysis, expressing confidence limits for the estimation of polymyxin B is a discrimination problem (6) rather than a prediction problem. Ninety per cent confidence limits are

TABLE IV.—EXAMPLE OF POTENCY DETERMINATION OF SAMPLES

	—Inhibition Zone Diameter, mm.—	
Plate	Reference 100 units polymyxin B, 20 mg. sucrose per ml.	Sample 1, 19 mg. sucrose per ml.
1	14.8	15.4
2	15.6	15.7
3	15.2	15.7
4	15.8	15.8
5	15.7	15.9
6	15.6	15.6
7	15.8	15.5
8	15.5	15.6
9	15.9	15.8
10	15.6	16.0
11	15.6	15.4
12	15.5	15.6
13	15.0	15.0
14	15.2	15.4
15	15.7	16.0
Average	15.50	15.63
Correction	−0.33	−0.33
Corrected Av.	15.17	15.30
Units/ml.		106

estimated to be at $X_1 \pm 0.0639$ or roughly $\pm 16\%$. Although this confidence interval appears large, it is acceptable for an antibiotic assay considering that a $\pm 10\%$ interval exists when polymyxin B alone is assayed (1), that only one average zone of inhibition is determined to estimate the polymyxin B potency when the interfering substance is present, and that a discrimination problem is involved. This procedure represents an improvement in accuracy over the common practice of not considering the sucrose effect.

DISCUSSION

The procedure discussed above presents a means of correcting for the interference of sucrose in the polymyxin B plate assay. Variations in the conduct of the assay in different laboratories may create significant differences in the regression coefficients although they are not expected. Significant differences of regression coefficients may be tested for by using the t test (2).

The nature of the multiple regression equation found indicates the type of interference caused by the presence of sucrose. Since the covariance of the sucrose regression coefficient with the intercept is

negative while the sucrose regression coefficient is positive, the over all sucrose effect might increase or decrease the zone diameter depending on the concentration present. Whether this produces any clinical advantage or disadvantage is unknown. No investigation has been made regarding sugars other than sucrose.

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Some Chemical Effects of Focused Ultrasound on Glutathione and other Compounds*

By RICHARD E. FAUST† and GUSTAV E. CWALINA‡

Hydrogen peroxide is produced in water exposed to focused ultrasound. Ultrasonic energy will oxidize potassium iodide and sodium bisulfite, alter certain colorants and indicators, and deaminate glycine. The oxidation of reduced glutathione is shown by the nitroprusside test, titration of residual sulfhydryl groups, and paper partition chromatography. Nearly one-half of the tripeptide is destroyed after exposure for forty-one minutes.

INSUFFICIENT KNOWLEDGE of the biological effects on tissue components hinders a more extensive use of ultrasonic energy in medicine. Although the production of heat within tissues exposed to acoustic energy is of vital significance in treating such conditions as rheumatism and arthritis, the concomitant cavitation that may occur at high energies cannot be ignored (1). For example, an electrical discharge at the moment of cavitation produces a photochemical decomposition of water with the formation of certain oxidizing species, such as oxygen, hydrogen peroxide, and the hydroperoxyl radical (HO_2) (2). Thus, cavitation with the production of oxidizing agents may be a primary factor when evaluating ultrasonic effects on tissue. Besides showing the oxidative potentialities of ultrasound on various chemicals, this study demonstrates the ultrasonic oxidation of the biologically important tripeptide, glutathione.

ULTRASONIC APPARATUS

The source of the ultrasound waves in this study is a Hypersonic Transducer Model BU-305, manufactured by the Brush Electronic Co. of Cleveland, Ohio. The transducer contains a concave bowl of barium titanate ceramic operating by means of the reverse piezoelectric effect at a resonant frequency of 400 kilocycles per second. Approximately 400 ml. of distilled water containing 1 ml. of 1% Aerosol OT® was used as the coupling fluid in all experiments. A cooling coil of copper tubing, connected with an electric pump and circulating ice water, aided in maintaining reduced temperatures in the coupling fluid and in the solution being insonated. The transducer was powered by a radio-frequency generator delivering a R. F. power of 250 watts maximum at a frequency variable from 100 to 1,000 kilocycles per second (3).

It is important to mention here the difference between our apparatus and that used by many workers in earlier studies of ultrasonic oxidation. To date most of the chemical effects of ultrasound have been evaluated using flat quartz crystals delivering 10 to 40 watts per square centimeter (4). Concave ceramic elements, similar to the one used in this study, are capable of focusing energies as high as 2,000 watts per square centimeter. Few studies of the chemical effects of high frequency sound waves have been performed at such high acoustic energies.

EXPERIMENTAL

A Study of the Oxidizing Agents Produced in Water by Ultrasound.—The formation of hydrogen peroxide in insonated water was demonstrated by the decoloration of potassium permanganate solutions and by the production of a yellow color with titanium sulfate solutions.

The addition of a few drops of freshly prepared benzidine solution (2% in glacial acetic acid) to insonated water caused the immediate production of a characteristic golden-yellow color that faded slowly upon standing. Direct insonation of water containing a few drops of benzidine test solution developed a similar yellow color. Insonated water that had been placed in a boiling water bath for thirty minutes still reacted to give the vivid yellow

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color. Even after boiling the insonated water for five minutes in a direct flame, a faint but distinct yellow color was noted. Since this characteristic golden-yellow color is not produced when benzidine test solution is added to dilute solutions of hydrogen peroxide, nitrous acid, or nitric acid, it is probably due to some relatively stable decomposition product of water.

The Effect of Ultrasound on Various Chemical Compounds.—Dilute solutions of several indicators including chlorphenol red, thymol blue, bromphenol blue, and phenol red showed color fading after insonation. The addition of either dilute acid or alkali failed to restore the color. Dilute solutions of several dyes and colorants including D. and C. Blue No. 4, F. D. and C. Red No. 4, F. D. and C. Yellow No. 5, carmine, and aniline green showed darkening and color modifications.

The oxidation of sodium bisulfite was studied and it was shown that the rate of oxidation is related to the acoustic intensity, the period of exposure, and the temperature.

Insonation of potassium iodide solution containing carbon tetrachloride gives a greater iodine release than does potassium iodide solution containing chloroform. Iodine liberation from pure potassium iodide solution is proportionately smaller. The oxidation of potassium iodide was more uniform and more abundant with special thin-walled, round-bottomed glass flasks than with ordinary test tubes.

The Effect of Ultrasound on Several Amino Acids.—It was decided to study the effect of ultrasound on two of the constituent amino acids of glutathione; glycine and cysteine, as well as cystine, an amino acid containing the disulfide linkage.

Glycine.—An experiment was performed in which definite quantities of Nessler's reagent were added to varying concentrations of dilute ammonia solution. By this procedure it was demonstrated that the rapidity with which a yellow cloudiness develops is dependent upon the ammonium ion concentration. Insonated glycine solutions developed the yellow turbidity in approximately one-half the time required for control solutions of the amino acid. The greater ammonium ion concentration is due to deamination of glycine by the acoustic energy.

Cystine.—If rupture of the disulfide bond in cystine occurs during insonation, then a positive nitroprusside test may be obtained. However, when 20 ml. of a 0.0325 *M* cystine solution in 1 *N* HCl was insonated for ten minutes at the highest intensity possible, it failed to show a coloration different from control solutions when tested with the nitroprusside reagent. No sulfate was detected with barium chloride test solution and no hydrogen sulfide was noted with lead acetate test solution or paper. Aqueous mixtures containing excess amino acid were insonated and tested for sulfhydryl groups, sulfate, and sulfide with negative results.

A paper chromatographic analysis of insonated cystine solutions was performed in order to detect possible decomposition products. Significant R_f values were obtained with isobutyric acid solvent (5) and with butanol-ethanol-hydrochloric acid solvent (5), but no essential difference was found in the R_f values or character of the spots in the insonated and control chromatograms. Examination under ultraviolet light before and after spraying with ninhydrin failed to elucidate additional spots.

Cysteine.—Lavine's technique (6), a procedure for titrating sulfhydryl groups in molar HI, was used to estimate the effect of ultrasound on the —SH group. When a 0.0325 *M* aqueous cysteine solution was insonated for ten minutes at the highest possible intensity in a thin-walled flask no destruction of —SH groups occurred. Tests for hydrogen sulfide with lead acetate test solution and paper were negative. The addition of barium chloride test solution failed to detect sulfate.

Control and insonated cysteine solutions (aqueous and 1 *N* HCl) were examined by ascending paper chromatography. Solvent solutions were phenol saturated with water, phenol-salt, and isobutyric acid (5). Developing solutions included ninhydrin (5), azide-iodine (7), potassium permanganate (8), and sodium nitroprusside (9). In all experiments no significant differences between the R_f value, size, and color of the various spots in insonated and control chromatograms were observed.

The Oxidation of Reduced Glutathione by Ultrasound.—The destruction of reduced glutathione by ultrasound has been demonstrated by the three following experimental procedures: the nitroprusside reaction, titration of the sulfhydryl groups, and paper partition chromatography.

The addition of a freshly prepared sodium nitroprusside solution and of ammonium hydroxide solution to insonated and control samples of reduced glutathione generated a red-violet color of varying intensity. The color intensity is related to the concentration of —SH groups. In more than a dozen tests, using reduced glutathione that had been exposed to various intensities of ultrasound for different time periods, the treated tripeptide gave pale violet colors and the control solutions displayed dark, red-violet colors.

The titration procedure of Woodward and Fry (10) was used for the determination of —SH groups in reduced glutathione. A special test tube target chamber, consisting of an "acoustic window" of thin plastic fitted over the open end of the tube and secured with elastic bands, was used in this phase of the work. Since ultrasound punctured the plastic window at the 110 intensity during fifteen-, twenty-eight-, and forty-one-minute exposures, it was necessary to use two thicknesses of plastic in these series. To prevent air pockets which would interfere with the transmission of the sound waves into the processing vessel, the two layers of plastic were joined together by a few drops of 1% Aerosol OT® solution.

After adding 22 ml. of reduced glutathione solution (0.05% in deionized water) to the test tube, the plastic "acoustic window" was firmly attached. The test tube was then immersed in an ice-water bath for ten minutes before insonation. During the insonation period the tube was raised or lowered slightly so that the wattage delivered to the titanate element was maintained at a maximum. This maximum reading was consistently accompanied by the production of abundant cavitation bubbles throughout the target solution and by only slight agitation of the target solution. After insonation the plastic membrane was quickly removed and the temperature of the solution recorded. Temperature

determinations during insonation were impossible, for it was shown that a mercury thermometer gave erratic, rapidly fluctuating readings when placed in the target solution. Tubes exposed to higher intensities and for longer periods of time increased in temperature up to 50° and were cooled to 25° before titration. Solutions of reduced glutathione heated in a water bath for forty-one minutes at 55° gave similar titration figures as nonheated samples, showing that the increase in temperature during insonation is not responsible for the destruction of sulfhydryl groups. At higher intensities and longer exposure periods the transducer element heats up rapidly causing its electrical impedance to rise, which in turn causes an accumulation of current in the generator circuit. This build-up of current activates a circuit breaker protecting the generator circuit and automatically cuts the power to the transducer. Therefore, during the readings at 110 for fifteen, twenty-eight, and forty-one minutes the rheostat frequently had to be lowered to 100 to prevent the circuit breaker from operating. Table I presents the percentages of glutathione destroyed as calculated from the average of four readings.

TABLE I.—DESTRUCTION OF REDUCED GLUTATHIONE BY FOCUSED ULTRASOUND

Intensity	Exposure Time, min.	Glutathione Destroyed, %[a]
40	2	4.3
40	15	19.4
40	28	27.0
40	41	37.2
75	2	3.4
75	15	18.2
75	28	33.4
75	41	41.3
110	2	3.6
110	15	23.4
110	28	37.2
110	41	48.3

[a] 0.05% in deionized water.

TABLE II.—PAPER CHROMATOGRAPHY OF GLUTATHIONE

Solution[a]	Procedure	First Spot *Rf*	Second Spot *Rf*
Insonated	Isobutyric acid solvent, dry 5 min. at 95°, ninhydrin	0.04	None
Control		0.04	0.24
Insonated	Isobutyric acid solvent, air dry 2 hrs., ninhydrin	0.05	None
Control		0.07	0.26
Insonated	Phenol-salt solvent, dry 5 min. at 95°, ninhydrin	0.10	None
Control		0.10	0.38

[a] 5 lambda spotted.

The ascending paper chromatographic techniques used in the study of insonated glutathione closely follow those used with the sulfur-containing amino acids, cystine and cysteine. Control chromatographic strips displayed two spots, the lower corresponding to oxidized glutathione and the upper to reduced glutathione. Strips prepared from insonated solutions of the tripeptide, however, presented a single spot corresponding to oxidized glutathione. In each experiment this single spot was larger and darker in color than the lower spot of the control, indicating a greater concentration of oxidized glutathione after insonation. Table II presents examples of data obtained in this chromatographic analysis. Two-dimensional chromatography using phenol-salt (5) and collidine-lutidine (5) solvents and ninhydrin developer failed to show other differences between control and insonated glutathione solutions.

SUMMARY

1. The presence of hydrogen peroxide in water exposed to focused ultrasound is demonstrated.

2. Color reactions with benzidine test solution indicate the presence of a relatively stable decomposition product in insonated water.

3. Various colorants and indicator solutions, potassium iodide, sodium bisulfite, and glycine are shown to be affected by exposure to focused ultrasound.

4. The destructive oxidation of reduced glutathione by ultrasound proceeds to at least the oxidized glutathione stage; and after exposure for forty-one minutes, nearly one-half of the tripeptide is destroyed.

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An *in Vitro* Evaluation of Some Hydrophilic Colloids as Bulking Agents*

By A. L. MONACO and E. J. DEHNER†

An evaluation of hydrophilic colloids as bulking agents based on comparative water-retention capacities determined under rigidly controlled conditions is presented. Included among the substances tested were typical natural, water-soluble gums and synthetic hydrophilic colloids. Both dry and liquid forms of the several test materials have been evaluated. Determinations of the water-retention capacities were made by the method of Blythe, *et al.*, with significant modifications. Data are presented, showing the effect of temperature, a hitherto relatively unconsidered factor, on water-retention.

PREVIOUS STUDIES, by other investigators, have shown the comparative water-retention capacities of various hydrophilic colloids at 25°, using a standardized osmometric procedure. The present study extends these investigations to include data indicating the effect of temperature and osmotic pressure variation on water-retention capacity. A modified osmometric apparatus and procedure are described.

This study was undertaken to extend the previously reported data relating to the comparative hydrophilic properties of standard bulk laxative materials. Both natural and synthetic colloids, as such, and in proprietary preparations were evaluated by a method which measures relative ability to retain water against the osmotic pull of a hypertonic solution across a semipermeable membrane. Significant modifications of a reported osmometric procedure, involving thermal and concentration factors, have been introduced to increase the generally accepted physiological significance of this type of bulking agent evaluation.

Primarily because of its obviously direct relationship to physiological process, relative simplicity, reproducibility of results, and secondarily in the interest of standardization of pharmaceutical testing techniques, the method of measurement chosen was essentially that of Blythe, *et al.* (1). This method is based upon the theory that the laxative value of a hydrophilic colloid is directly related to its ability to hold water against adsorption from the intestinal tract into the blood stream, water being deemed essential to the formation of a stool of adequate bulk and desirable consistency.

The hydrophilic colloid types of bulk laxatives have definite advantages over other types of laxatives (2). In the dispersed state, they lack harsh and irritating properties and act as emollients, coating and lubricating the intestinal wall (3). Their water-binding capacities aid in increasing bulk which stimulates peristaltic movement while maintaining the softness essential to easy defecation. Moreover, the stimulus to intestinal movement is relatively mild, though usually effective, and less conducive to development of the "laxative habit."

Recent clinical investigations have amply demonstrated the superiority of the newer synthetic hydrophilic colloids over the naturally occurring vegetable materials (4–9). The former are nontoxic and nonantigenic (10, 11). The synthetic products are cleaner, more uniform, and are available in varying grades permitting consistent reproduction of a wide range of gel strengths. Specifically, the methylated celluloses are nonmetabolized (10, 13), completely egested (14), and possess outstanding water-retentive capacities (1).

The experiments reported herein were undertaken to demonstrate further the reported superiority of the synthetic colloids over the natural products and to ascertain the relative values of the bulk laxative and high gel grades of methylcellulose[1], with respect to water retentive capacity. The modifications of the method of Blythe, *et al.* (1), involved the use of a redesigned osmometric apparatus, variations in the concentrations of dispersed colloid, use of a solution of increased hypertonicity and measurements at both room temperature and normal body temperature.

Materials tested included proprietary preparations containing karaya gum and psyllium seed and products containing several viscosity types of methylcellulose in solid and liquid dosage forms.

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[1] Methocel HG 400, Dow Chemical Co.

Test Procedure.—A series of test mixtures was prepared in which the colloid was dispersed in sufficient Artificial Intestinal Solution[2] to make one liter of 1.0, 1.5, or 2.0 per cent w/v dispersions of the active bulking constituent of the product being tested. An Eppenbach Homo Mixer was used at moderate speed for ten minutes to insure a thorough dispersion. Each dispersion was allowed to stand for twenty-four hours, to effect complete hydration of the colloid, and then deaerated. A volume of 600 cc. of this deaerated mixture (B) was poured into a glass battery jar[3] (A) (Fig. 1). An osmometer cell[4] (D), fitted with two 600 APT cellophane membranes[5] with an effective diameter of 56.6 mm. per membrane, was filled with a hypertonic solution (C) containing either 30 per cent w/v or 50 per cent w/v Carbowax® 4000 W[6] dispersed in water. A 400 mm. column (E) with a side arm was attached to the cell by a section of tubing (H). This assembly was placed in the battery jar (A) containing the test mixture (B), exercising care to avoid entrapping air around either membrane. The battery jar was covered with aluminum foil to minimize contamination and evaporation of the test mixture. Additional hypertonic solution was introduced through the top end of the column until it began to fall from the side arm. The overflow liquid (G), composed of a mixture of hypertonic solution and water pulled from the dispersion, was collected in a 100-ml. graduated cylinder (F). This overflow volume was recorded at twenty-four and forty-eight-hour intervals. If at any time of observation, the overflow exceeded 60 cc., the quantity was recorded and the graduate emptied. The volume of the dispersion in the battery jar was adjusted if great water loss warranted it. All tests were run in duplicate with Artificial Intestinal Solution used as a control. (Fig. 2.)

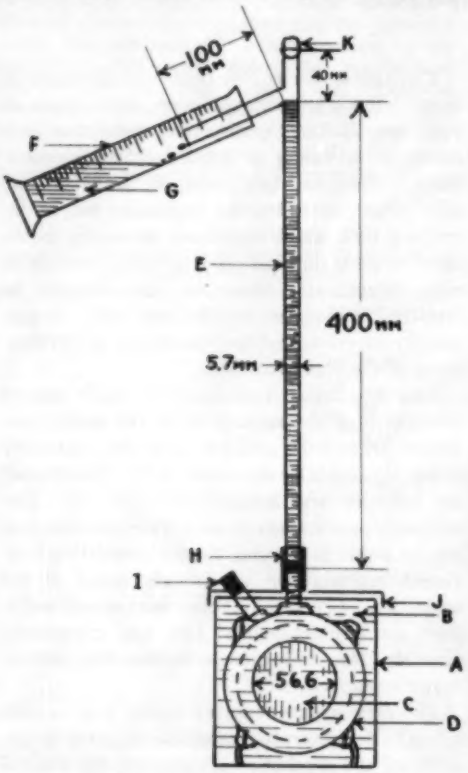

Fig. 1.—Apparatus for determination of water retention against osmotic pull, measured by water loss.

A—Battery jar, 4 in. x 4 in. x 4 in., A. H. T. Co. No. 2098
B—Test mixture
C—Hypertonic solution
D—Osmometer, Cenco Troxel, Cen. Sci. Co. No. 74157
E—Manometer tube
F—Graduate, 100 ml.
G—Overflow from osmometer
H—Rubber hose connection
I—Rubber stopper, No. 00
J—Aluminum foil cover, A. H. T. Co. No. 1038
K—Cotton plug

[2] Formula for Artificial Intestinal Soln.: Pancreatin, 0.28%; Bile Salt, 1.00%; Methylparaben, 0.18%; Propylparaben, 0.02%; Sodium Bicarbonate, 1.50%; and Distilled Water *q. s.*, 100.0%.
[3] Battery jar 4" x 4" x 4" A. H. Thomas No. 2098.

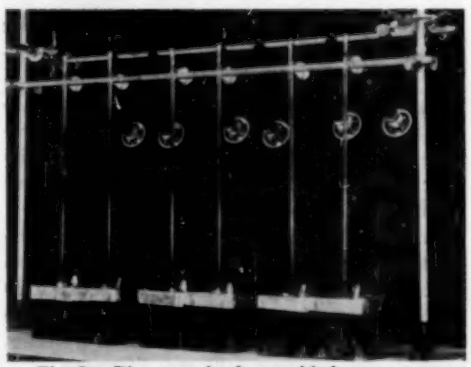

Fig. 2.—Photograph of assembled apparatus.

EXPERIMENTAL

Series I.—For a general evaluation, all tested substances (Table I) were subjected to osmometric study in a dispersed concentration of 2% active bulking constituent versus a 30% w/v hypertonic solution, at room temperature (25°). The viscosity of the test mixture was measured with the Brookfield Viscometer, to determine the possible correlation to water-retentive properties. The *p*H was determined, using the Beckman *p*H Meter, to

[4] Osmometer Cell, Cenco Troxel, Cen. Sci. Co. No. 74157.
[5] Du Pont Cellophane.
[6] A form of polyethylene glycol.

TABLE I.—2% ACTIVE CONSTITUENT VERSUS 30% HYPERTONIC SOLUTION AT 25°

	Product Ingredient	Form	Amount Used per Liter	Brookfield Viscosity Determination: Spindle	r. p. m.	Viscosity, cp.	pH	Water Loss in cc.: 24 hr.	48 hr.
A	20%[a] Methylcellulose	Susp.	100 cc.	2	10	655	7.75	30	46.5
B	100%[a] Methylcellulose	Gran.	20 Gm.	2	10	170	8.03	40.5	61
C	0.5 Gm.[b] Methylcellulose	Tab.	40 Tabs.	2	10	150	7.93	42	64
D	80% Karaya gum	Gran.	25 Gm.	4	4	5000	7.93	44	67
E	100% Psyllium seed	Flakes	20 Gm.	4	20	4060	7.80	46.5	70
F	50% Psyllium seed	Gran.	40 Gm.	4	20	2600	7.95	51	75
G	... Artif. intest.	Soln.	...	1	20	5	8.06	110	166

[a] Grade unknown—probably BL Grade. [b] Tablets crushed before dispersion.

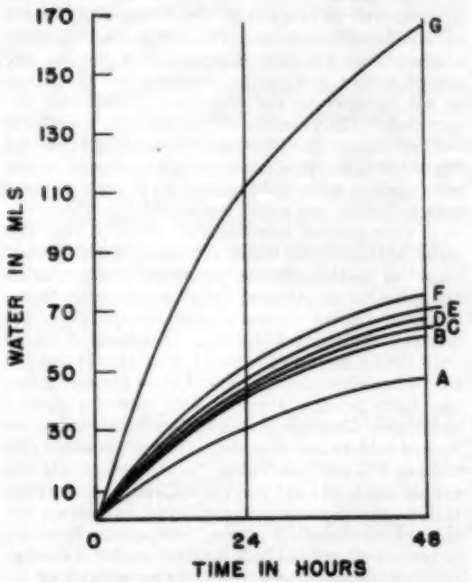

Fig. 3.—Series I—water loss rate of 2% colloid dispersed in A.I.S. versus 30% Carbowax® at 25°.

observe any significant alteration of the pH of the intestinal solution induced by an effective dose of the bulking agent.

Series II.—A more specific study was made to determine the comparative retentive capacity of two commercially available liquid preparations containing methylcellulose (one, a 9% dispersion of a low viscosity grade, the other, a 20% suspension of a bulk laxative grade) and a formulation, developed by the authors, containing 20% of a high gel grade in suspension. The tests were run with 1% w/v concentration of active bulking agent versus 30% w/v hypertonic solution at room temperature (25°).

Series III.—Variations in concentration of the two preparations in *Series II*, containing 20% suspended colloid, were studied. The concentrations tested included 1.0%, 1.5%, and 2.0% of dispersed colloid versus 30% hypertonic solution. The room temperature, during the time in which this series was run, was 33°.

Series IV.—The relatively higher water losses found at 33°, as compared with 25°, led to the comparison of the retentive capacity of the two prepara-

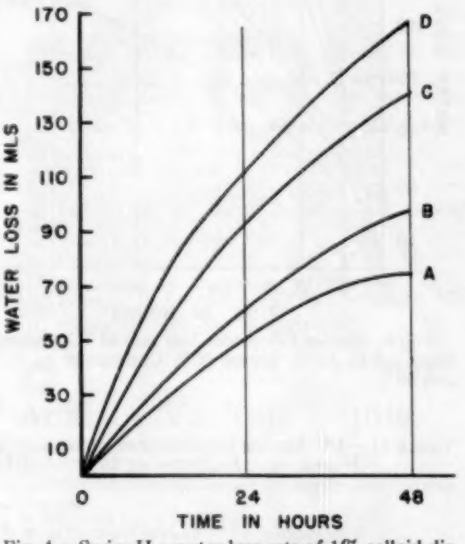

Fig. 4.—Series II—water loss rate of 1% colloid dispersed in A.I.S. versus 30% Carbowax® at 25°.

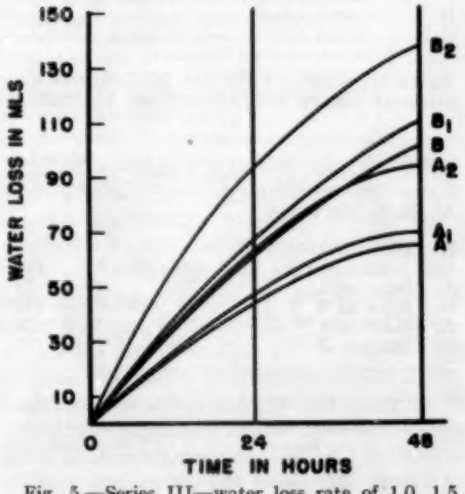

Fig. 5.—Series III—water loss rate of 1.0, 1.5, and 2.0% colloid dispersed in A.I.S. versus 30% Carbowax® at 33°.

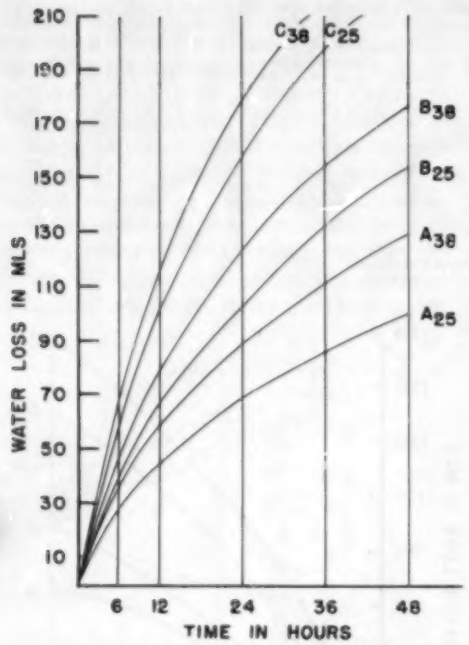

Fig. 6.—Series IV—water loss rate of 1% colloid dispersed in A.I.S. versus 50% Carbowax® at 25° and 38°.

TABLE II.—1% ACTIVE CONSTITUENT VERSUS 30% HYPERTONIC SOLUTION AT 25°

		Product Ingredient	Amount Used per Liter, cc.	Water Loss in cc. 24 hr.	48 hr.
A	20%	Methocel® HG	50	51	74
B	20%	Methocel® BL	50	61	96
C	9%	Methocel® appr. 15 cp.	111.1	92	140
D	...	Artif. intest.	...	110	166

TABLE III.—1.0%, 1.5%, AND 2.0% ACTIVE CONSTITUENT VERSUS 30% HYPERTONIC SOLUTION AT 33°

	Product Ingredient	Amount Used per Liter, cc.	Water Loss in cc. 24 hr.	48 hr.
A	20% Methocel® HG	100	43	65
B	20% Methocel® BL	100	61	101.5
A_1	Same as A	75	47	70
B_1	Same as B	75	67.5	110.5
A_2	Same as A	50	63.5	93.5
B_2	Same as B	50	94	138

tions used in *Series III* at room (25°) and body temperature (38°). The greater water loss noted in *Series III* diluted the hypertonic solution and diminished its osmotic activity, therefore, a solution of higher tonicity (50% w/v Carbowax® 4000W in water) was used to compensate for this dilution effect. For purposes of simplicity the test was run with 1% w/v active component. Readings at six, twelve, twenty-four, thirty-six and forty-eight-hour intervals were required due to the increased volume of the overflow.

Discussion.—The results obtained from *Series I* (Table I, Fig. 3) confirm the work done by Blythe (1), in that the products containing the synthetic, methylcellulose, were superior in water-retentive properties to the proprietary items containing karaya gum and psyllium seed. The *p*H of the dispersion medium was not altered appreciably by the dispersed colloid and further observations of change in *p*H induced by the dispersed colloid were not recorded. The viscosity of the test mixture offered neither direct nor reciprocal correlation to the water-retentive properties of the colloids except when dealing with modifications of the same colloidal entity, hence, continued studies of this characteristic were deemed unnecessary. Results from this series indicated the water retention of the "liquid form" of methylcellulose to be better than either the granular or tableted "dry" form probably because of higher viscosity grade Methocel in the former. Subsequent testing, therefore, involved only the liquid form (Table II, Fig. 4). It was felt that a liquid preparation would yield a superior dosage form because there is less tendency toward esophageal blockage, which has been reported in the case of tablets and granules. The preparation containing 9% methylcellulose, in the completely dispersed state, did not display sufficient water retention, in the concentration employed, to warrant further comparison. A direct comparison involving only the high gel and bulk laxative grades of methylcellulose demonstrated a definite superiority of the former type, at varying concentrations. High gel grade methylcellulose dispersions showed less water loss at 1% concentration than did the bulk laxative grade at 2% concentration (Table III, Fig. 5). Increased water losses noted in this series were attributed to the higher room temperature (33°) prevailing during the course of the experiment. The higher water losses observed at 33° prompted the initiation of the experiment to determine water losses at normal body temperature (38°), as a closer approach to *in vivo* conditions. In anticipation of a greater dilution factor the concentration of Carbowax® 4000 W, in the hypertonic solution, was increased to 50%.

Results (Table IV, Fig. 6) indicate that the water loss of the dispersion of high gel grade methylcellulose at 38° is less than the loss from the dispersion of the bulk laxative grade at 25°, a further demonstration of the superiority of the former.

TABLE IV.—1% ACTIVE CONSTITUENT VERSUS 50% HYPERTONIC SOLUTION AT 25° AND 38°

		Product Ingredient	Water Loss in cc. at 25° 6 hr.	12 hr.	24 hr.	36 hr.	48 hr.	Water Loss in cc. at 38° 6 hr.	12 hr.	24 hr.	36 hr.	48 hr.
A	20%	Methocel® HG	28	45	66	86	100	36	59	89	112	129
B	20%	Methocel® BL	40	67	103	133	154	46.5	79	123	154	176
C		AIS control	58.5	101	157.5	198	225	67	116	178	218	243

SUMMARY

1. A modified osmometric procedure has been used to compare the water-retentive properties of some proprietary preparations containing psyllium seed, karaya gum, and several grades of methylcellulose. The results confirm the work of previous investigators (1).

2. Products containing the synthetic hydrophilic colloid, methylcellulose, were superior in water-retentive properties to products containing natural hydrophilic colloids. Dispersions of methylcellulose of relatively low viscosity showed greater water-retentive capacity than equally concentrated, natural gum dispersions of greater viscosity. The latter behavior may be clinically significant in minimizing the probability of intestinal blockage or impaction.

3. Liquid preparations of low viscosity, bulk laxative, and high gel grades of methylcellulose were compared. High gel grade methylcellulose was found to possess superior water-retention capacity, in all tests performed.

4. A modified osmometric apparatus is described.

5. Differences in water-retentive capacities are more clearly defined when determined at 38°.

6. Measurement of water retention against a solution of increased hypertonicity (50% Carbowax® 4000 W) at body temperature (38°) constitutes, we believe, a closer approach of the test method, to physiological conditions. The great dehydrating capacity of the colon is best simulated by a solution possessing a high degree of hypertonicity.

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The Stability of Ascorbic Acid in Various Liquid Media*

By F. J. BANDELIN and J. V. TUSCHHOFF

The stability of ascorbic acid at several concentrations in various liquid media including aqueous solutions of cellulose gums, pectin, sucrose, dextrose, corn syrup, and in glycerin and propylene glycol was studied. The effect of *p*H in aqueous solutions of ascorbic acid was also studied. Viscosity alone as produced by gums did not prevent decomposition of ascorbic acid. Sugar and sorbitol solutions exerted a marked stabilizing effect. Ascorbic acid was relatively stable in glycerin and propylene glycol and in sugar and sorbitol solutions. Data are presented on the rates of decomposition of ascorbic acid in these media after storage at room temperature and at 40° C. The stability of ascorbic acid in syrups containing conjointly vitamins of the B-complex is not materially affected.

THE TENDENCY OF ASCORBIC ACID to decompose in solution has been a distinct deterrent to its use in liquid pharmaceutical preparations. Although methods of stabilizing aqueous solutions of ascorbic acid, usually as the sodium salt for parenteral use, have been discussed in the literature (1–14) little has been published concerning its stability in liquid preparations for oral administration. Giral (15), in 1947 published a study of factors affecting the destruction of ascorbic acid solutions. While the work of the present paper was in progress, a paper by Bartilucci and Foss (16) touched upon the subject of ascorbic acid stability in various mixtures of liquid media incidental to a study of the stability of vitamin B_{12} solutions containing ascorbic acid.

To our knowledge, no comprehensive, systematic, or integrated study of the stability of ascorbic acid, in usual therapeutic concentrations, in various liquid media, has appeared. We therefore undertook to study the effects of *p*H, oxidation, dilution, and various compounds commonly encountered in pharmaceutical liquids such as ethanol, sugars, certain polyhydric alcohols, and natural and synthetic gums. It is the purpose of this paper to present the scope, details, and observations, of this study.

* Received August 28, 1954, from the Research Laboratories of Flint, Eaton & Co., Decatur, Ill.

TABLE I.—PER CENT ASCORBIC ACID REMAINING IN SOLUTIONS AT VARIOUS pH LEVELS AFTER AERATION AT 25°C.

Hrs.	Ascorbic Acid, 1 mg. per ml.				Ascorbic Acid, 5 mg. per ml.			
	pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
0	100	100	100	100	100	100	100	100
1	96.0	90.4	86.2	82.4	97.7	93.2	90.8	88.2
2	88.4	80.6	70.4	68.7	90.6	84.0	77.6	74.3
3	84.5	75.6	61.4	56.3	88.7	78.2	66.4	62.5
4	82.2	68.8	50.3	45.2	87.1	72.9	56.4	54.2
5	80.2	61.4	39.2	32.9	83.7	68.5	47.7	46.4
6	77.5	56.2	30.4	23.4	80.5	65.5	42.0	37.4
7	72.2	51.7	24.4	16.3	76.8	61.7	32.6	30.2
24	62.0	39.9	12.0	0	71.1	48.7	23.8	6.0

TABLE II.—PER CENT ASCORBIC ACID REMAINING IN VARIOUS SOLUTIONS AFTER AERATION AT 25° C.

Hrs.	Ascorbic Acid, 5 mg./ml. in:											
	25% Ethanol	25% Propylene Glycol	25% Glycerin	25% Sucrose	25% Sorbitol[a]	25% Corn Syrup[a]	50% Ethanol	50% Propylene Glycol	50% Glycerin	50% Sucrose[a]	50% Sorbitol[a]	50% Corn Syrup[a]
0	100	100	100	100	100	100	100	100	100	100	100	100
2	96.5	99.5	100	93.5	100	94.7	100	99.5	99.5	99.5	99.5	99.4
4	96.5	99.5	99.6	92.5	96.5	94.3	99.3	99.2	99.5	99.5	97.8	95.0
7	95.0	94.2	95.0	89.4	95.4	94.4	97.4	96.5	99.5	98.6	97.8	95.0
24	80.0	91.0	90.5	79.5	89.7	88.4	92.2	96.0	97.1	96.5	97.0	93.7

[a] w/v solutions based on solids content; others, all liquids, prepared on v/v basis.

EXPERIMENTAL

Solutions of ascorbic acid of various selected concentrations and at definite *p*H levels were prepared in distilled water and in various liquid media as hereinafter described. Syrups were also prepared with synthetic vitamins of the B-complex. Solutions were observed after aging at room temperature and at 40° and some after aeration for varying periods of time. Analyses were carried out at definite intervals using the official dichlorophenol-indophenol method after appropriate dilution. In all cases, the quantity of ascorbic acid found on analysis immediately after preparation of the solution was considered as 100%.

Effects of pH and Aeration.—Solutions containing 1 and 5 mg. per ml., respectively, of ascorbic acid were prepared and buffered to *p*H 3.0, 4.0, 5.0, and 6.0 using 0.5 *N* acetic acid and 0.5 *N* sodium acetate. Two hundred milliliters of solution was placed in each of four 250-ml. cylindrical graduates and air passed through them at a constant rate of 120 bubbles per minute. Residual ascorbic acid in the solutions was determined after one, two, three, four, five, six, seven, and twenty-four hours. Results are given in Table I. These results indicate that the oxidative destruction of ascorbic acid is accelerated as the acidity of the solution decreases. The solution containing 5 mg. of ascorbic acid per ml. showed a smaller percentage loss than did the weaker solution. After seven hours, loss was accelerated. The solutions having a *p*H of 6 acquired a yellow color after two hours of aeration while those having a *p*H of 5 acquired a yellow color after three to four hours of aeration. Solutions with *p*H 3 and *p*H 4 remained clear and water-white over the entire twenty-four hour period.

Effect of Aeration on Solutions Containing Ethanol, Polyhydric Alcohols, and Sugars.—Solutions containing 5 mg. per ml. of ascorbic acid were prepared in 25 and 50% aqueous solutions each of ethanol, propylene glycol, glycerin, sucrose, sorbitol, and corn syrup. These solutions have a *p*H of approximately 3.0.

Two hundred milliliters of each solution was aerated as previously described in a 250-ml. cylindrical graduate and analyses carried out after two, four, seven and twenty-four hours. Results are given in Table II.

All compounds in both concentrations exerted a noticeable stabilizing effect on ascorbic acid. The protective action of sucrose was considerably better at the higher concentration. Propylene glycol, glycerin, sucrose, and sorbitol all seemed to afford about the same amount of protection in 50% concentration.

Effect of Aging at 40°.—In addition to the alcohol and sugar vehicles already investigated, additional ascorbic acid solutions were prepared with synthetic and natural gums. These included methylcellulose, carboxymethylcellulose, pectin and tragacanth. These gums were used to impart viscosity to the solutions with the thought that viscid solutions might hinder oxygen exchange, thereby producing a stabilizing effect upon the dissolved ascorbic acid. Solutions containing 5 mg. and 20 mg. of ascorbic acid per ml. were prepared and kept at 40° for twelve weeks. Analyses were carried out at two-week intervals. Results are given in Table III.

The gums investigated offered no protection to the decomposition of ascorbic acid and, in fact, seemed to accelerate its decomposition when compared against control solutions of ascorbic acid in distilled water.

In this series, sugars and polyhydric alcohols again exhibited a marked stabilizing effect upon ascorbic acid solutions of both concentrations. Glycerin, sorbitol, U. S. P. Syrup, and propylene glycol appear to possess the greater stabilizing effect on ascorbic acid in solution.

TABLE III.—STABILITY OF ASCORBIC ACID IN VARIOUS LIQUID MEDIA AT 40° C.

Solvent	Ascorbic Acid, 5 mg. per ml. — Weeks						Ascorbic Acid, 20 mg. per ml. — Weeks					
	2	4	6	8	10	12	2	4	6	8	10	12
1. Corn syrup[a]	96.5	93.5	91.0	86.5	83.5	82.0	97.5	94.0	93.0	88.0	84.5	83.0
2. Sorbitol[b]	99.5	99.5	98.5	94.0	91.5	90.0	99.5	99.5	99.5	95.0	92.5	90.0
3. 4% CMC Med. vis.[c]	79.5	72.0	56.0	36.0	14.0	..	86.0	74.5	63.0	53.5	47.5	44.5
4. Glycerin	98.0	96.0	96.0	93.0	90.5	87.0	100	100	99.5	98.5	95.0	93.0
5. Propylene glycol	95.5	92.0	87.0	82.0	72.0	63.0	100	100	98.0	93.5	90.0	84.0
6. 3% Methylcellulose 1500 c.p.s.	78.0	69.0	50.5	33.0	15.0	..	95.0	88.5	77.0	70.5	58.5	55.0
7. 4% Pectin	76.0	53.0	39.0	13.0	..	..	94.0	86.0	79.5	77.5	55.0	48.0
8. Syrup U. S. P.	99.5	97.5	94.5	90.0	90.0	88.0	99.0	98.5	94.5	92.5	91.5	90.0
9. Syrup 425 Gm./L.	97.0	91.0	80.0	72.5	53.0	44.0	99.0	98.0	90.0	81.0	77.5	73.0
10. Syrup 212 Gm./L.	92.0	74.0	70.5	63.5	41.0	33.0	93.5	92.0	86.5	72.0	67.5	65.0
11. Corn syrup 50% in water	95.5	86.0	77.5	68.0	63.0	59.5	97.0	93.0	80.0	75.5	68.0	62.0
12. 2.5% Tragacanth solution	72.0	41.0	27.0	10.0	..	..	88.0	70.0	59.5	52.0	41.5	37.0
13. Sat. soln. dextrose	94.5	89.0	87.5	84.0	81.0	77.0	95.5	92.0	90.0	87.5	86.0	83.5
14. Distilled water	78.0	68.0	56.0	39.0	28.0	..	94.0	86.0	75.0	68.0	59.0	53.0

[a] Corn Syrup used marketed as Sweetose by the A. E. Staley Co., Decatur, Ill.
[b] Arlex is a solution containing approximately 83% sorbitol, obtainable from Atlas Powder Co., Wilmington, Del.
[c] Carboxymethyl cellulose, sodium salt, medium viscosity.

TABLE IV.—PER CENT ASCORBIC ACID REMAINING IN SOLUTIONS ON STORAGE AT ROOM TEMPERATURE (5 MG. PER ML. ASCORBIC ACID)

Solvent	Days						
	30	60	90	120	180	240	360
1. Corn syrup	96.5	92.5	92.0	92.0	90.0	86.0	76.0
2. Sorbitol	99.0	99.0	99.0	97.0	96.0	92.5	89.0
3. 4% CMC Med. vis.	84.0	68.0	56.5	38.0	..	..	..
4. Glycerin	100	100	99.0	99.0	97.0	93.5	92.0
5. Propylene glycol	99.5	99.0	98.0	94.5	92.0	90.0	90.0
6. 3% Methylcellulose 1500 c.p.s.	76.0	60.0	39.5	15.0	..	..	..
7. 4% Pectin	80.5	78.0	64.5	56.0	46.0	26.0	..
8. Syrup U. S. P.	100	100	98.0	98.0	93.0	90.0	88.0
9. Syrup 425 Gm./L.	98.0	96.0	94.5	94.0	86.5	79.0	69.0
10. Syrup 212 Gm./L.	88.0	81.0	77.5	74.5	64.5	59.0	44.0
11. Corn syrup 50% in water	96.0	88.0	87.0	76.5	75.0	68.0	56.0
12. 2.5% Tragacanth solution	78.5	62.0	51.0	32.0	..	..	..
13. Sat. soln. dextrose	99.0	93.5	87.5	80.0	64.0	58.0	51.0
14. Distilled water	90.0	81.5	74.5	67.5	40.5	18.5	..

TABLE V.—STABILITY OF VARIOUS CONCENTRATIONS OF ASCORBIC ACID IN WATER, PROPYLENE GLYCOL, AND U. S. P. SYRUP AT ROOM TEMPERATURE, PER CENT ASCORBIC ACID REMAINING IN SOLUTION

Ascorbic Acid	Days						
	30	60	90	120	180	240	360
10 mg./ml. water	93.0	84.0	82.0	67.0	51.5	41.0	..
50 mg./ml. water	94.0	92.0	88.0	79.5	60.5	59.0	30.0
100 mg./ml. water	97.0	93.0	91.0	83.5	70.5	68.0	59.0
10 mg./ml. P. G.	100	98.5	98.0	97.5	96.0	92.0	86.0
50 mg./ml. P. G.	100	97.0	98.0	98.0	98.0	96.5	93.5
100 mg./ml. P. G.	100	100	100	100	99.0	100	92.5
10 mg./ml. Syrup	100	100	98.0	99.0	97.0	96.0	84.0
50 mg./ml. Syrup	100	100	100	100	99.0	100	96.0
100 mg./ml. Syrup	100	100	100	100	100	100	99.5

Effect of Aging at Room Temperature.—Solutions listed in Table III were aged at room temperature at the 5 mg. per ml. level. Results of aging over a period of three hundred and sixty days are given in Table IV. In this series as in the previous one aged at 40°, glycerin, propylene glycol, sorbitol, and U. S. P. Syrup again appeared superior to the other media. In both series, it was noted that reduction of the sugar content of the vehicle was accompanied by a corresponding increase of ascorbic acid destruction. Also in group 4, gums, both natural and synthetic, accelerated the rate of destruction of ascorbic acid compared to an aqueous solution under the same conditions.

Effect of Concentration.—Since ascorbic acid appeared to be relatively stable at room temperature

in propylene glycol and in U. S. P. Syrup, solutions were prepared containing 10, 50 and 100 mg. per ml., respectively, in each of these and in water. Propylene glycol may be considered to represent an anhydrous solvent while syrup is an aqueous solution. Control solutions of each of these concentrations were made up in distilled water and all were allowed to age at room temperature for three hundred and sixty days with analyses for ascorbic acid being carried out at regular intervals. Results are given in Table V.

In all cases higher concentrations of ascorbic acid showed less relative destruction based on the per cent of residual ascorbic acid.

Stability of Ascorbic Acid in Syrups Containing B-Complex Vitamins.—Syrups containing ascorbic acid with synthetic vitamins of the B-complex were prepared in a base of U. S. P. Syrup and in sorbitol (Arlex). Results of aging at room temperature and at 40° are given in Table VI. These preparations had a *p*H ranging from 3.1 to 3.4. Under conditions of the test, ascorbic acid in conjunction with vitamins of the B-complex seemed to be more stable than in syrups containing no other vitamins. Loss of ascorbic acid, even after two years, was less than 15% in all cases.

TABLE VI.—ASCORBIC ACID IN VITAMIN B-COMPLEX SYRUPS AT ROOM TEMPERATURE AND AT 40° C.

		Ascorbic Acid Remaining in Solution, %					
		Days				Years	
		90	180	270	360	2	3
Sucrose base[a]	RT	100	100	98	98	88	80
	40°	98	94	84	76	..	..
Sorbitol base[b]	RT	100	98	98	94	86	78
	40°	97	92	84	72	..	..
Sucrose base[c]	RT	100	99	94	92	89	82
	40°	95	92	80	68	..	..
Sorbitol base[d]	RT	100	98	95	91	87	70
	40°	96	92	84	62	..	..

[a] Composition: Each ml. contains: ascorbic acid 20 mg., thiamine hydrochloride 0.6 mg., riboflavin 0.4 mg., niacinamide 6 mg., U. S. P. Syrup *q. s.*

[b] Same composition as *a* except Arlex *q. s.*

[c] Each ml. contains: ascorbic acid 6.25 mg., thiamine hydrochloride 0.25 mg., riboflavin 0.25 mg., pyridoxine hydrochloride 0.25 mg., calcium pantothenate 0.5 mg., U. S. P. Syrup *q. s.*

[d] Same components as *c* except Arlex *q. s.*

DISCUSSION

The stability of ascorbic acid in solution is greatly dependent upon *p*H. In the presence of oxygen, ascorbic acid is easily oxidized to dehydroascorbic acid. Although this reaction is reversible, under certain conditions, and the dehydro form exhibits all the activity of the hydro form, the dehydro form is further decomposed to soluble, biologically inactive compounds. This latter reaction is irreversible and may take place even in the absence of oxygen if the solution is at *p*H 4.0 or higher. The rate of oxidation of ascorbic acid is accelerated with increasing *p*H, actual decomposition (caramelization) taking place above *p*H 7.2. Aeration of ascorbic acid solutions definitely indicates that the rate of oxidative decomposition of ascorbic acid increases as the *p*H value increases. The addition of certain alcohols and sugars as described exerts a noticeable protective action on ascorbic acid by retarding oxidative destruction. This may be due to the reduced ability of these solutions to carry dissolved oxygen since increasing concentrations of these compounds in the solutions produces increased stability of the ascorbic acid. That the viscosity of the solution is probably not a factor in so far as ascorbic acid stability is concerned is indicated by the fact that various gums in solution, producing increased viscosity, did not seem to retard the decomposition of ascorbic acid but, in fact, seemed to accelerate it.

Syrups of ascorbic acid containing 1 and 5 mg. of ascorbic acid per ml. (which may be considered within the usual therapeutic range for pharmaceutical preparations) are relatively stable for short periods of time or even up to one year. U. S. P. Syrup, sorbitol, glycerin, and propylene glycol were all of approximately equal efficacy in retarding the destruction of ascorbic acid solutions on aging.

The inclusion of vitamins of the B-complex to syrups of ascorbic acid seemed to produce a stabilizing effect. Whether or not this is an artifact or due to reaction of some of the ascorbic acid with some of the vitamin bases such as the reaction of ascorbic acid with niacinamide described by Fox and Paterson (17) is, as yet, a matter of conjecture. At this point, most evidence suggests that ascorbate ion is more prone to decomposition than is the undissociated acid.

SUMMARY

1. The rate of oxidative decomposition of ascorbic acid solutions increases as the *p*H value increases. Destruction of the acid is markedly accelerated above *p*H 4.0.

2. Addition of ethanol, polyhydric alcohols such as glycerin, propylene glycol, and sorbitol, and sugars such as sucrose, corn sugar, and dextrose, all tend to produce a stabilizing effect on ascorbic acid. Sucrose, sorbitol, glycerin, and propylene glycol are superior to the others in this respect.

3. Vegetable gums added to produce solutions of greater viscosity seem to accelerate destruction of the vitamin.

4. Syrups and solutions of ascorbic acid in varying concentrations indicate that higher concentrations of ascorbic acid are relatively more stable than are lower concentrations.

5. Vitamin B-complex factors added to syrups of ascorbic acid in U. S. P. Syrup and in sorbitol seem to increase the stability of ascorbic acid.

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The *in Vitro* Effect of Sodium Lauryl Sulfate on Vaginal Pathogens*

By JAMES E. O'BRIEN and RICHARD K. THOMS

Sodium lauryl sulfate *per se* and in combination with a sodium perborate formulation has been tested against organisms typical of those occurring in the flora associated with *Trichomonas vaginalis* vaginitis and Monilial vaginitis. Our results indicate that both sodium lauryl sulfate and the sodium perborate formulation are effective agents against vaginal pathogens but they are not synergistic.

THE ANTIMICROBIAL PROPERTIES of sodium lauryl sulfate are well known (1–3). It has been applied effectively in combination with sodium perborate as a douche in vaginal infections for several years and recently other formulations have come into use (1).

To date there have been no quantitative studies on the activity of sodium lauryl sulfate alone and in combination with sodium perborate against the specific organisms involved in vaginitis. This has left in doubt the most effective concentration of sodium lauryl sulfate and whether there is any synergistic action in the combination with the sodium perborate.

To measure these actions, an *in vitro* method was required which would be applicable to *Candida albicans, Streptococcus pyogenes, Micrococcus pyogenes* var. *aureus,* and *Trichomonas vaginalis* with no more variation than absolutely necessary to suit their individual growth requirements in order that comparable results could be obtained.

Since bactericidal techniques which require the drug to kill in a given time introduce speed of action as a variable between organisms, a simple stasis technique was employed in which the concentration of the drug to produce 100 per cent inhibition was determined.

EXPERIMENTAL

Microorganisms.—*Candida albicans* and *Trichomonas vaginalis* have been well established as the causative agents of Monilial vaginitis and *Trichomonas vaginalis* vaginitis (4–8). *Streptococcus pyogenes* and *Micrococcus pyogenes* var. *aureus,* while occurring in the normal vaginal flora and in Monilia and Trichomonad infections, are also possibly the causative agents of nonspecific leukorrhea (9). Doderlein's bacillus which has been shown to be identical with *Lactobacillus acidophilus* is found in the normal flora and is either lacking or found to a lesser extent in the infectious states mentioned (10).

Pathogenic strains of *Candida albicans, Micrococcus pyogenes* var. *aureus,*[1] and *Streptococcus pyogenes*[1] were obtained in pure culture, as well as a strain of *Lactobacillus acidophilus,* for use in this study. A bacteria-free strain of *Trichomonas vaginalis* was obtained by the method of Johnson, Trussel, and Jahn (11) from a patient exhibiting all of the known clinical manifestations of *Trichomonas vaginalis* vaginitis.[2]

METHODS

C. albicans, S. pyogenes, M. pyogenes var. *aureus,* and *L. acidophilus.*—The media used for maintenance and tests were those considered optimal for each organism. They are listed in Table I.

TABLE I.—MEDIA USED

Organism	Medium
C. albicans	Sabouraud liquid medium (Difco)
S. pyogenes	Brain heart infusion medium (Difco)
M. pyogenes var. *aureus*	Brain heart infusion medium (Difco)
L. acidophilus	Micro-inoculum broth (Difco)
T. vaginalis	Simplified trypticase serum medium (12)

TABLE II.—THE SODIUM LAURYL SULFATE DOUCHE FORMULA

	Per Cent in Dry Formula	mg./L. in Douche Solution
Sodium perborate	66.0	9,900
Sodium borate	33.0	4,950
Sodium lauryl sulfate	1.0	150

Standard inocula were prepared by suspending the organisms in sterile distilled water and adjusting the light transmittance by means of a Lumetron to 80% for *C. albicans* and 85% for the other three organisms.

Tubes containing 10 cc. of the appropriate medium to which various concentrations of the drugs had been added in a standard volume of 1 cc. were inoculated with 0.1 cc. of the distilled water suspension of organisms. Control tubes containing

* Received October 10, 1954, from the School of Pharmacy, University of Connecticut, Storrs.

Abstracted from a thesis submitted to the Graduate School of the University of Connecticut by James E. O'Brien in partial fulfillment of the requirements for the degree of Master of Science.

This project was supported by a grant from the Fesler Company of Stamford, Connecticut.

Presented to the Scientific Section, A. PH. A., Boston meeting, 1954.

[1] The authors are indebted to Dr. Robert C. Cleverdon, University of Connecticut, for supplying these cultures.

[2] The authors are indebted to Dr. R. Baltrucke, St. Francis Hospital, Hartford, Conn., for supplying this inoculum and establishing its virulence.

TABLE III.—THE EFFECT OF THE DRUGS ON THE TEST ORGANISMS

Organism	Agent	Concn. range in final experiment, mg./L.	Increment, mg./L.	Minimum Concn. (mg./L.) Causing 100% Inhibition
C. albicans	Sodium perborate formulation	17.0 to 208.0	40.0	166.6
	Sodium lauryl sulfate	8.5 to 17.0	1.5	13.3[a]
	Sodium perborate formulation 25 mg./L.-Sodium lauryl sulfate	1.7 to 25.0	3.0	13.3[a]
S. pyogenes	Sodium perborate formulation	48.2 to 83.0	1.7	83.0
	Sodium lauryl sulfate	33.3 to 158.3	1.7	129.5
	Sodium perborate formulation 48.2 mg./L.-Sodium lauryl sulfate	33.3 to 158.3	3.5	132.5
M. pyogenes var. *aureus*	Sodium perborate formulation	3.3 to 18.3	1.7	16.7
	Sodium lauryl sulfate	1.7 to 25.0	1.7	21.7
	Sodium perborate formulation 5.0 mg./L.-Sodium lauryl sulfate	5.0 to 25.0	1.5	20.4
L. acidophilus	Sodium perborate formulation	26.7 to 90.0	10.0	90.0
	Sodium lauryl sulfate	1.7 to 16.7	1.7	8.3
	Sodium perborate formulation 26.7 mg./L.-Sodium lauryl sulfate	1.7 to 16.7	1.7	8.3
T. vaginalis	Sodium perborate formulation	9.0 to 22.9	1.7	19.5
	Sodium lauryl sulfate	0.9 to 16.7	1.8	8.0
	Sodium perborate formulation 17 mg./L.-Sodium lauryl sulfate	0.9 to 16.7	1.8	8.0

[a] This is the minimum concentration producing 50% inhibition. No greater inhibition could be obtained in reasonable concentrations.

10 cc. of medium and 1 cc. of sterile distilled water were inoculated in the same manner. These tubes were then incubated with shaking at 37.5° for twelve hours. At the end of this time the amount of growth was ascertained by turbidity determinations.

All concentrations and controls were run in duplicate tubes. The blanks used in the evaluations were incubated with the inoculated tubes. The average turbidity of the two tubes was converted to per cent inhibition by means of the following formula:

$$100 - \left(\frac{96 - T_1}{96 - T_2}\right) \times 100 = \text{per cent inhibition}$$

T_1 is the average turbidity of the treated tubes
T_2 is the average turbidity of the control tubes
96 is the turbidity of the tubes immediately following inoculation. This was always the same and may be regarded as a constant.

Trichomonas vaginalis.—The medium used for this organism is given in Table I. Tubes containing 10 cc. of this medium to which various concentrations of the drugs had been added in a standard volume of 1 cc. were inoculated with 300,000 organisms taken from forty-eight-hour cultures adjusted to contain this number in 0.3 cc. These tubes were then incubated without shaking at 35° for forty-three hours. Growth was determined by cell counts using a hemocytometer. All concentrations of the drugs and controls were run in duplicate.

EXPERIMENTAL PLAN

The sodium lauryl sulfate douche formulation is given in Table II.

The concentrations of this formula, omitting the sodium lauryl sulfate, to produce 100% and 0% inhibition were determined as were those of sodium lauryl sulfate alone. To accomplish this it was first necessary to determine the range. Final experiments were then run over a narrow range using small increments. The concentration ranges and the increments used in those ranges as given in Table III are those used in the final experiments. The final experiments with sodium lauryl sulfate were repeated using the maximal concentrations of the sodium perborate-sodium borate mixture which had no effect on the organisms together with the various concentrations of sodium lauryl sulfate. The purpose of this was to determine if a synergistic action could be shown.

RESULTS AND DISCUSSION

The results obtained in this study are given in Table III. It will be noted that the concentration to cause 100% inhibition are almost the same for sodium lauryl sulfate alone and in combination with the maximal concentration of the sodium perborate-sodium borate mixture which had no effect. This indicates that no synergism exists.

It will also be noted that in the case of every microorganism except *Candida albicans*, both agents were effective in concentrations below those used clinically (Table II). In all cases at least one concentration was well below that used clinically. This indicates that from the standpoint of antimicrobial efficiency there is no theoretical reason to change the concentration of sodium lauryl sulfate in the formulation.

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A Pharmacological Investigation of the Seeds of *Pyrularia pubera* Michx.*

By JOSEPH P. BUCKLEY, SAMUEL T. COKER, and ROSE GOLDFIELD

Pharmacological studies were made of a partially purified extract of the seeds of *Pyrularia pubera*. Tests showed the extract to be a powerful, smooth muscle stimulant, especially on the uterus and isolated intestinal strips of the guinea pig and rabbit.

WIECZORKOWSKI AND DARBAKER reported that minute doses of a fluidextract of the whole fruit and of the kernel of *Pyrularia pubera* were lethal to guinea pigs (1). Danti administered 1-cc. doses of an aqueous and an alcoholic extract from the pericarp to frogs and concluded that these extracts produced no pronounced pharmacological activity on the frog (2). Goldfield administered varying doses of the fixed oil obtained from the seed, orally and intravenously, to rats and found that the oil produced no apparent pharmacological effect (3).

Since very little information had been published concerning the pharmacology of *Pyrularia pubera* and since several sources had reported the presence of a toxic substance or substances in the fruit (1, 4, 5), the authors believed that a pharmacological investigation of the defatted seeds was indicated.

EXPERIMENTAL

Preparation of the Partially Purified Extract.—The fruit was collected in the Ohiopyle region, Fayette County, Pa., in September, 1951.[1] The seeds were decorticated, air-dried for forty-eight hours, and comminuted in a Waring Blendor. The ground seeds were then defatted with petroleum ether. A fluidextract was prepared from the defatted material by process A of the U. S. P. using a menstruum of 70% ethyl alcohol and 30% distilled water. An aliquot portion of the extract was then diluted with an equal part of distilled water producing a yellowish precipitate, which in turn was removed by extracting with chloroform. This resulting hydro-alcoholic solution could be diluted at least ten times with distilled water without evidence of a precipitate being formed. This 50% extract which was used for the pharmacological investigation contained an equivalent of 500 mg. of the defatted ground drug per cc.

PHARMACOLOGICAL PROPERTIES

Rabbit Blood Pressure and Electrocardiographic Studies.[2]—Rabbits of either sex were anesthetized with a mixture of 40 mg. of pentobarbital sodium and 250 mg. of urethane per Kg. administered intraperitoneally. Minute doses of the extract, administered into the femoral vein, produced no significant change in the blood pressure until the toxic dose was reached, at which point there was a drop in the blood pressure and a marked increase in the depth of respiration. Electrocardiograms obtained from lead II indicated some delay in conduction, bradycardia, bigeminal patterns, and ventricular tachycardia (Fig. 1). The approximate minimal lethal dose in the rabbit was found to be 0.1 cc./Kg. intravenously.

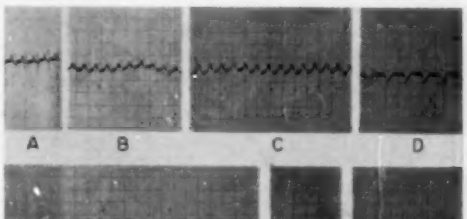

Fig. 1.—Electrocardiographic recordings obtained from Lead II on a 2-Kg. rabbit: A - normal; B and C - records after administering 0.025 cc. of the 50% extract; D, E, F, and G - records after administering a total dose of 0.2 cc. of the 50% extract.

Action on the Isolated Rabbit Heart.—Oxygenated Locke's solution, prepared with Water for Injection, was perfused through rabbit hearts according to the Langendorff procedure at a pressure of 40 mm. of Hg. The temperature of the perfusate as it entered the heart was between 38 and 39°.

* Received November 15, 1954.
From the School of Pharmacy, University of Pittsburgh, Pittsburgh, Pa. Presented to the Scientific Section, A. PH. A., Boston meeting, August, 1954.
[1] The authors are indebted to Drs. E. C. Reif and R. Sager for their collection and to Dr. E. P. Claus for his identification of the fruit.
[2] The authors wish to thank Dr. Sidney G. Odle for his interpretation of the electrocardiograms.

Doses of 0.01 cc. of the 50% extract injected into the coronary inflow cannula increased ventricular tone. Administration of larger doses further increased the tone but decreased the rate and height of the contraction. A dose of 0.01 cc. also decreased the coronary flow by 34.3% (range 30–38%).

Action on the Turtle Heart in Situ.—Turtle Ringer's was perfused through turtle hearts in the usual manner. A dose of 0.06 cc. of the extract injected into the venous cannula produced a positive tonotropic effect, bradycardia, and arrhythmia. Large doses produced systolic arrest (Fig. 2).

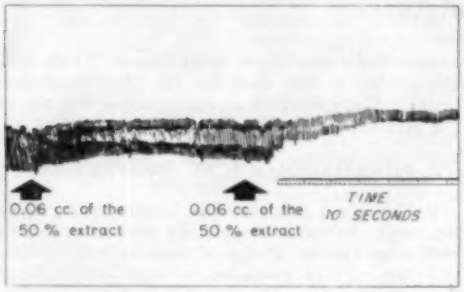

Fig. 2.—Effects of the extract on the turtle heart *in situ*.

Action on the Isolated Uterus.—Guinea-pig and rabbit uteri, approximately 2.5 cm. in length, were suspended in 70 cc. of oxygenated Locke's solution maintained at 38°. Doses ranging between 0.02 cc. and 0.1 cc. produced a marked increase in the tone of both the pregnant and nonpregnant uterus (Fig. 3).

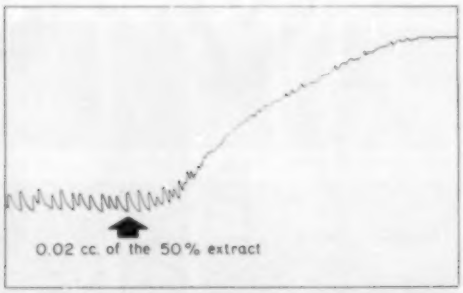

Fig. 3.—Effects of the extract on the guinea pig uterus *in vitro*.

Rat Uterus in Situ.—Albino rats, approximately 200 Gm., in early or late estrus, were anesthetized with 1.2 Gm./Kg. of urethane administered intraperitoneally. One uterine horn was isolated and protected by a glass enclosure containing mineral oil warmed to 38°. A 0.025 cc./Kg. dose of the extract injected into the femoral vein produced a marked increase in the height of the contraction and a slight increase in the tone (Fig. 4). The animals died within ten minutes.

Isolated Intestines.—Strips of guinea-pig and rabbit ileum were suspended in 70 cc. of Locke's solution as per Magnus. A dose of 0.02 cc. of the extract produced an increase in the rate of contractions, a marked increase in the tone, and a decrease in the height of the contractions (Fig. 5). These effects also occurred in preparations previously treated with two drops of 1:1000 atropine sulfate solution.

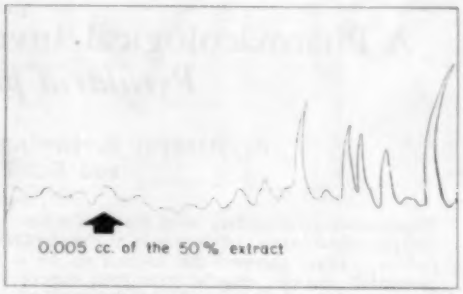

Fig. 4.—Effects of the extract on the rat uterus *in situ*.

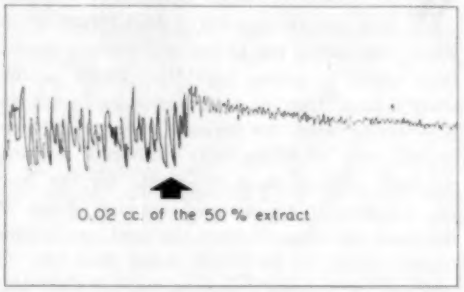

Fig. 5.—Effects of the extract on the ileum of the guinea pig *in vitro*.

Frog Hind Leg Perfusion.—The hind legs of frogs (*R. pipiens*) were perfused according to the method of Trendelenburg (6). Normal perfusion rates in drops per five-minute intervals were taken. Doses of the extract ranging from 0.01 cc. to 0.1 cc. were injected into the arterial cannula and the drops per five-minute intervals were again taken. The average decrease in the rate of flow produced by 0.01 cc. of the extract was 44.5% ± 23.7%.

Acute Toxicity in Rats.—One hundred to 150 Gm. albino rats were fasted overnight prior to use. Six groups containing five animals per group were employed and doses of the 50% extract ranging from 0.02 cc. to 0.1 cc./Kg. were administered via intraperitoneal injection. The animals were kept at 25–30° in individual cages for twenty-four hours after which time the number of dead animals were counted. The LD_{50} calculated by the De Beer graphic method (7) is 0.092 cc./Kg.; limits of error, 92–109%.

Small doses of the extract produced slight depression. Larger doses produced ataxia, principally involving the hind legs, dyspnea, and mydriasis.

SUMMARY AND CONCLUSIONS

These studies on a fluidextract prepared from the seeds of *Pyrularia pubera* indicate the pres-

ence of an extremely toxic principle. The LD_{50} of the 50 per cent extract, intraperitioneally in the albino rat, is 0.092 cc./Kg. and the minimal toxic dose is approximately 0.1 cc./Kg. intravenously in the rabbit. The extract is a powerful, smooth muscle stimulant. This action is especially demonstrated on isolated guinea-pig and rat uteri and on guinea-pig and rabbit intestinal strips. It has positive tonotropic effect on the ventricle and appears to be particularly toxic to the myocardium.

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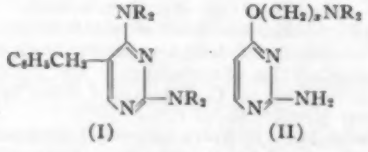
Basically Substituted 5-Benzylpyrimidines*

By F. LEONARD, H. HORN, F. E. ANDERSON, and A. GOLDBERG

A number of substituted 5-benzylpyrimidines have been prepared for pharmacological evaluation. None showed activity in screening tests for broncholytic, hypotensive, or oxytocic activity.

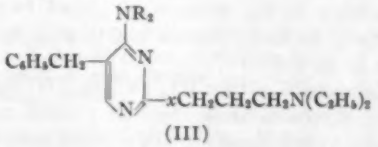
MOST OF THE PHARMACOLOGICAL DATA on pyrimidine derivatives which is now in the literature was obtained either as a by-product of metabolic studies on thiamin and its hypothetical precursors, or from investigations in the fields of antibacterial and antimalarial agents (1). Several chemical investigations in recent years were, however, motivated by interest in the actions of pyrimidine derivatives on the central and autonomic nervous systems. Goldberg (2) reported in 1951 the results of a synthetic program on 5-benzyl-2,4(disubstituted-amino) pyrimidines of the general formula I.



(I) (II)

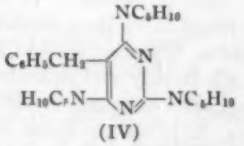
One of these, 5-benzyl-2,4-bis-dimethylaminopyrimidine [I, $NR_2 = N(CH_3)_2$] proved to be an especially effective antagonist of barbiturate narcosis while 5-benzyl-2,4-bis-(1-piperidyl)-pyrimidine (I, $NR_2 = NC_5H_{10}$) was shown to be a powerful central nervous system depressant. Patents were issued to Oroshnik in 1952 on a series of 2-amino-4-dialkylaminoalkoxypyrimidines (II) which, it was alleged, possessed oxytocic and hypotensive properties (3).

Acid addition salts of compounds of type I are very slightly soluble in water and form acid solutions. It was thought that, (*a*) the interposition of a polymethyleneoxy or polymethyleneamino group between the basic groups and the ring (compounds of type III), and (*b*) the addition of a third basic moiety to the ring would yield bases capable of forming more nearly neutral, more soluble acid addition salts than those obtained from I. III were additionally of interest for their relation to II.



(III)

NR_2 = 1-piperidyl-, cyclohexylamino-, 2-furfurylamino.



(IV)

A number of 5-benzyl-2-chloro-4(substituted-amino)pyrimidines were available to us from a previous investigation (2). These gave when refluxed, (*a*) with sodium 3-diethylaminopropanol in 3-diethylaminopropanol as a solvent, the basic alkylethers (III, $x = O$) and, (*b*) with excess 3-diethylaminopropylamine, the diamines (III, $x = NH$) described in the table below.

The synthesis of a 5-benzyl-2,4,6-(trisubstituted amino)pyrimidine was achieved starting from 5-benzylbarbituric acid which was converted on refluxing with phosphorous oxybromide to 5-benzyl-2,4,6-tribromopyrimidine which when heated with piperidine for twenty-four hours in a sealed tube at 160° gave 5-benzyl-2,4,6-

* Received December 15, 1954, from the Research Laboratories of Nepera Chemical Co., Inc., Yonkers, N. Y.

tri(1-piperidyl)pyrimidine(IV). This method of preparation of IV was satisfactory for small samples but impractical for larger quantities because phosphorous oxybromide is not commercially available.

The beneficial effect of phenol in displacement reactions has been known for some time. Bartlett and Danben (4) reported the marked effect of phenol in promoting ether formation from triphenylmethyl halides. Surrey and Cutler (5) found that in the presence of phenol, reaction occurred readily between 2-chloro-, 4-chloro-, and 4,7-dichloroquinoline and secondary amines but proceeded to a negligible extent in its absence. Phillips (6) noted the more favorable yields of 2-amino-6-methyl-4-(1-piperidyl) pyrimidine which were obtained from the action of piperidine on 2-amino-4-chloro-6-methylpyrimidine in the presence of phenol than in its absence. The role of phenol in these reactions has been rationalized (6) on the basis of the "push-pull" concept of polar displacement reactions which was elaborated by Swain (7). Thus, it is thought that phenol exerts a solvolytic pull on the halogen atom which augments the push of the nucleophilic amine. We found that 5-benzyl-2,4,6-trichloropyrimidine reacts with piperidine in the presence of phenol as satisfactorily as does 5-benzyl-2,4,6-tribromopyrimidine in its absence.

Compounds of type III (x=O or NH) did not yield crystalline salts but all formed water-soluble hydrochlorides giving sensibly neutral reactions when added to a hydrochloric acid solution containing one equivalent of hydrochloric acid. The basic properties of IV on the other hand were found to be little greater than those of I, for like I, it yielded an acid addition salt which formed strongly acid aqueous solutions.

The compounds described herein were evaluated by Drs. R. J. Schachter and E. Kimura in the Department of Pharmacology of these Laboratories and found to be devoid of broncholytic, hypotensive, or oxytocic activity. We are indebted to Dr. T. Fand and Miss R. Becker of the Analytical Division of this company for the microanalyses reported herein, and to Miss E. Gelblum for technical assistance.

EXPERIMENTAL

The following examples illustrate the procedures employed for the synthesis of the basic alkyl ethers and substituted trimethylenediamines reported in the table below.

5-Benzyl-2-(3-diethylaminopropoxy)-4-(1-piperidyl)-pyrimidine.—Sodium (0.634 Gm., 0.0275 Gm.-atom) was dissolved with heating at 80° and stirring in 50 ml. of 3-diethylaminopropanol. The solution was cooled to 50° and treated with a solution of 7.19 Gm. (0.025 mole) of 5-benzyl-2-chloro-4-(1-piperidyl) pyrimidine (2) dissolved in 20 ml. of 3-diethylaminopropanol. The resulting mixture was heated at 120-130° for four hours. The precipitate of sodium chloride was removed by suction filtration and washed with alcohol. The filtrate and washings were concentrated at water pump pressure and the residue fractionated.

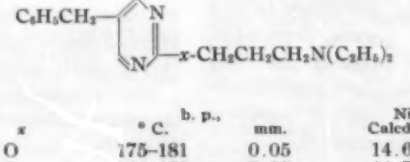
5-Benzyl-2-(3-diethylaminopropylamino)-4-(2-furfurylamino)-pyrimidine.—5-Benzyl-2-chloro-4-(2-furfurylamino) pyrimidine (2) (5.40 Gm., 0.02 mole) were dissolved in 25 ml. of 3-diethylaminopropylamine and the resulting solution refluxed for five hours. The mixture was cooled, made strongly alkaline with 10 N sodium hydroxide, and extracted with ether. The ether extracts were dried over anhydrous potassium carbonate and fractionated.

5-Benzyl-2,4,6-tribromopyrimidine. 5-Benzyl barbituric acid (5 Gm.) and 50 Gm. of phosphorus oxybromide (8) were heated together at 150–170° for twenty hours. The cooled reaction mixture was poured slowly into an ice-ether mixture. The etheral layer was removed, the aqueous layer extracted with ether, combined with the original ether layer and dried. Evaporation of the ether left a residue of 10 Gm. of crude tribromide. After recrystallization once from an acetone-water mixture (charcoal) and twice from methyl alcohol there remained 4.3 Gm. of material m. p. 105°.

Anal.—Calcd. for $C_{11}H_7Br_3N_2$: N, 6.88; Br, 58.9. Found: N, 6.83; Br, 57.1.

5-Benzyl-2,4,6-tri-(1-piperidyl) pyrimidine.—*Method A.* 5-Benzyl-2,4,6-tribromopyrimidine (4

TABLE I.—2,4-DISUBSTITUTED-5-BENZYLPYRIMIDINES



R	x	b. p., °C.	mm.	Nitrogen, % Calcd.	Found	ϕH 10^{-2} Molar Sol. of Mono HCl Salt
1-Piperidyl	O	175–181	0.05	14.6	14.8	6.04
2-Furfurylamino	O	184–186	0.05	14.7	14.3	5.93
Cyclohexylamino	O	185–190	0.05	14.2	14.7	5.87
1-Piperidyl	NH	215–217	0.1	18.0	17.9	6.47
2-Furfurylamino	NH	225–228	0.1	17.8	17.6	...
Cyclohexylamino	NH	226–229	0.2	17.6	18.4	6.47

Gm.) and 20 Gm. of freshly distilled piperidine were heated in a sealed tube, first at 100–110° for forty-eight hours, then at 160° for twenty-four hours. The cooled reaction mixture was treated with 100 ml. of 10% sodium carbonate and extracted four times with ether. Evaporation of the solvent and recrystallization of the crude product from acetone (charcoal) gave 2.2 Gm. of 5-benzyl-2,4,6-tri-(piperidyl)pyrimidine, m. p. 129–130°.

Anal.—Calcd. for $C_{26}H_{37}N_5$: N, 16.7. Found: 17.2.

Method B.—A mixture of 5 Gm. of 5-benzyl-2,4,6-trichloropyrimidine (9), 23 Gm. of piperidine and 8.3 Gm. of phenol was heated in a sealed tube at 150° for forty-eight hours. The reaction mixture (cool) was poured onto ice, made strongly acid with hydrochloric acid and extracted with ether to remove phenol. The aqueous acid solution was basified and the organic base extracted with ether. Evaporation of the ether left a residue of the desired triamine which after recrystallization from acetone, later from isopropyl alcohol, melted at 129–131°; yield 3.0 Gm.

Anal.—Calcd. for $C_{26}H_{37}N_5$: N, 16.7. Found: N, 17.2.

A monohydrochloride was prepared by addition of one equivalent of standardized ethereal hydrogen chloride solution to an ethereal solution of the base: m. p. 205–207°.

Anal.—Calcd. for $C_{26}H_{38}ClN_5$: Cl, 7.76. Found: Cl, 7.67.

A 1% solution of the hydrochloride in water has a *p*H of 1.78.

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Recently Developed Granulating Agents and Their Effect on Disintegration*

By JOHN C. GRIFFIN and C. LEE HUYCK

Carboxymethylcellulose, methylcellulose, sodium alginate, and extract of Irish moss, can be employed as granulating agents for compressed tablets of sodium bicarbonate and calcium gluconate. The agents studied proved effective in concentrations of 1 per cent excepting methylcellulose which required a concentration of 5 per cent. The disintegration time of the tablets studied compared favorably with starch paste when used with calcium gluconate; however, the disintegration time of sodium bicarbonate tablets granulated with starch paste proved to be much less than that of the other sodium bicarbonate tablets granulated with the agents under study. The mean square deviation of disintegration times in seconds of the various batches of tablets varied from 4 to 14 per cent.

THE SELECTION of a granulating agent for the preparation of compressed tablets has in the past often been a choice of one of the several time honored granulating agents such as syrup, gelatin solution, starch paste, etc. The purpose of this study is to evaluate several recently developed chemicals as granulating agents for tablets of soluble and insoluble medicaments.

Methylcellulose (1–3) compared favorably with starch paste and gelatin solution as a granulating agent for tablets. The disintegration of the tablets of various medicinals granulated with methylcellulose compared favorably with tablets of the same medicinals granulated with starch paste and gelatin solution.

Holstius and DeKay (4) investigated tablets containing sulfathiazole, sodium bicarbonate, and aspirin compound prepared according to the same formula. Gelatin-acacia solution, sucrose solution, and starch paste were used as granulating agents. Disintegration times were determined and submitted to a three-way statistical analysis to ascertain what factors significantly affected the rate of disintegration. Of the variables investigated such as active constituent, binding agent, and disintegrating agent, it appeared that no one variable was solely responsible for influencing the disintegration rate of the finished tablet. Disintegration is probably due to the interaction of the granulating agent, disintegrating agent, and active ingredient.

In a study of the influence of the degree of compression on the disintegrating properties of sulfathiazole tablets, Higuchi, *et al.* (5), concluded that disintegration time has little relation to

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granule size but it bears an exponential relationship to compressional force and it is reproducible.

EXPERIMENTAL

Sodium bicarbonate was chosen as the soluble medicinal agent to be tableted. Batches of 7000 tablets were prepared according to the following formula:

Sodium bicarbonate	5 lbs.
Talc	0.1 lb.
Heavy mineral oil	0.6 to 1 oz.
Granulating solution, a sufficient quantity.	

The granulating agents chosen for examination were as follows: *a*. Carboxymethylcellulose 70 D high (cellulose gum) (CMC), *b*. Methylcellulose 25 cps. (Methocel®), *c*. Sodium alginate (Kelgin®), *d*. Purified extract of Irish moss (Gelloid® 50), *e*. Corn starch (control).

With the exception of the control, duplicate batches of tablet granulations identified as A and B were prepared.

(*a*) A 1% solution of CMC[1] was prepared using cold distilled water and a high-speed agitator. One pound of 1% solution was required to moisten the sodium bicarbonate sufficiently to make a granulation which when dried would not fall to a fine powder when passed through a No. 16 screen. The moistened mass was granulated by the use of the Colton Wet Granulator provided with a No. 8 screen. The granules were spread on perforated trays covered with paper and dried for one hour at 49°, followed by overnight at room temperature exposed to air. The dry granules were passed through the Stokes Oscillating Granulator provided with a No. 16 screen. (*b*) Five per cent Methocel®[2] was mixed with one-fifth of the total required amount of distilled water. The mixture was heated to 87° and agitated until complete solution was effected. The remainder of cold water was added and the solution was thoroughly mixed. One- and two-tenths pounds of solution was necessary to prepare a satisfactory granulation. The granulation was prepared as described under CMC *a*. (*c*) A 1% solution of Kelgin®[3] was prepared using cold distilled water and high-speed agitation. One pound of solution was necessary to produce satisfactory results. The granulation was prepared as described under CMC *a*. (*d*) A 1% solution of Gelloid®[4] 50 was prepared with cold distilled water and agitation. One pound of solution was necessary to produce satisfactory results. The granulation was prepared as described under CMC *a*. (*e*) A control granulation was prepared with 10% corn starch in distilled water as granulating agent. A paste was prepared by mixing 1/10 lb. of starch with enough distilled water to make 1 lb. of mixture. The mixture was heated until the characteristic translucent appearance of the mass was noted. Eight-tenths pound of starch paste was required to granulate the sodium bicarbonate. The granulation was prepared as described under CMC *a*.

Before the dry granulations were compressed, each granulation was lubricated with a mixture of 1/10 lb. of talc and 2/3 to 1 oz. heavy mineral oil. The Stokes single punch Model F Tablet Machine provided with standard concave 13/32 inch punch and die was used for compressing the tablets. The average weight of the tablet was 5.2 gr. furnishing an average of 5 gr. sodium bicarbonate per tablet. An effort was made to compress all the tablets to 7 Kg. hardness when tested with the Monsanto Hardness Tester.

In order that a comparison be made of the effectiveness of the granulating agents for both soluble and slightly soluble medicaments, calcium gluconate was chosen as the slightly soluble medicinal. The formula used was as follows:

Calcium gluconate	5 lbs.
Lactose	2.99 lbs.
Sucrose	2.99 lbs.
Talc	0.16 lb.
Granulating solution, a sufficient quantity.	

The above batch size produced 14,000 tablets each weighing on an average of 5.6 gr. and containing 2.5 gr. calcium gluconate. The calcium gluconate was mixed with lactose and sucrose in a Hobart Mixer using number one slow speed mixing. The granulating agents in strengths previously specified were added gradually until granulations of proper consistency were obtained. The quantity of granulating agent added was kept constant at 1 lb. The granulations were prepared as previously specified for sodium bicarbonate. Punches (13/32 in.) and die were used for the compression of the tablets to a hardness of 7 Kg. when measured on the Monsanto Hardness Tester. A control tablet was prepared with starch paste in the same manner as described for the sodium bicarbonate tablet.

Disintegration tests were conducted according to the procedure outlined in the U. S. P. XIV (6). Data on the disintegration of the sodium bicarbonate and calcium gluconate tablets are given in Tables I and II, respectively.

TABLE I.—AVERAGE DISINTEGRATION TIME[a] IN SECONDS OF A AND B BATCHES OF SODIUM BICARBONATE TABLETS

	A	B	Mean
CMC®	648	673	660
Methocel®	708	711	710
Kelgin®	573	586	580
Gelloid®	705	709	707
Starch	43.5	40	42

[a] Average of six U. S. P. Disintegration Tests.

TABLE II.—AVERAGE DISINTEGRATION TIME[a] IN SECONDS OF A AND B BATCHES OF CALCIUM GLUCONATE TABLETS

	A	B	Mean
CMC®	987	1,018	1,003
Methocel®	548	575	562
Kelgin®	754	710	732
Gelloid®	662	662	662
Starch	533	495	514

[a] Average of six U. S. P. Disintegration Tests.

[1] CMC, cellulose gum brand of carboxymethylcellulose was obtained from the Hercules Powder Co., Wilmington, Del.

[2] Methocel®, brand of methylcellulose was obtained from The Dow Chemical Co., Midland, Mich.

[3] Kelgin®, brand of sodium alginate was obtained from the Kelco Co., 20 N. Wacker Drive, Chicago 6, Ill.

[4] Gelloid®, brand of purified extract of Irish moss was obtained from Jacques Wolf and Co., Passaic, N. J.

Statistical Study of the Disintegration Time of Tablets.—An average (7) gives considerable information about numerical data, but it does not show how the data varies or how much they differ from the average. A measure of dispersion or variance is therefore of considerable aid in summarizing the data.

To get a measure of dispersion from the deviation one must use the mean squares of the deviation. This quantity is called the variance of the set of variates. The variance however is not of the same dimension as the variates. The square root of the variance is measured in the same units as the variates and it is the measure of the dispersion which will be used in this report. It is called the standard deviation and is denoted by the symbol σ_x expressed symbolically as:

$$\sigma_x = \sqrt{\frac{\Sigma x_i^2}{n} - M_x^2}$$

The standard deviation as a means of measuring dispersion for a bell-shaped distribution curve is contained in the following:

A. Approximately two-thirds of the variates lie in the range of M equals $\pm \sigma$. B. Approximately 1% of the variates lie outside of the range of M plus or minus 3 σ.

The mean square deviation of the disintegration time in seconds of *A* and *B* batches of sodium bicarbonate and calcium gluconate tablets is given in Table III.

TABLE III.—MEAN SQUARE DEVIATION OF THE DISINTEGRATION TIME IN SECONDS OF A AND B BATCHES OF SODIUM BICARBONATE AND CALCIUM GLUCONATE TABLETS

	Sodium Bicarbonate		Calcium Gluconate	
	A-Batch	B-Batch	A-Batch	B-Batch
CMC®	83	54	102	46
Methocel®	51	45	14	33
Kelgin®	65	63	49	32
Gelloid®	28	32	32	55

The range of deviation for tablets of both soluble and slightly soluble medicaments containing the same granulating agent shows that the experimental error ranges from 4 to 13%.

DISCUSSION

The effective concentrations of the granulating solutions were 1% excepting methylcellulose which was used in 5% concentration. The quantities of solution used in each case to effect granulation should not be considered as a critical value for it is possible to effect granulation with smaller or larger amounts depending upon such conditions as the manner of mixing, the medicinal agent, and the diluent.

The sodium bicarbonate tablets compressed from the Methocel® and Gelloid® granulations were white, nonporous, smooth, and possessed a slight sheen characteristic of the talc and mineral oil lubricant. The sodium bicarbonate tablets compressed from the starch, Kelgin®, and CMC granulations possessed a like appearance but had a tendency to split when shaken in the bottle or between the palms of the hands.

The calcium gluconate tablets compressed from the CMC, starch, and sodium alginate granulations were white, nonporous, and smooth and possessed a slight sheen characteristic of the talc heavy mineral oil lubricant. The calcium gluconate tablets compressed from the Gelloid® and Methocel® granulations were white but were slightly porous and did not possess the slight sheen characteristic of the talc mineral oil lubricant.

The results of this study are in general agreement with previous studies (1, 4, 5). The applicability of any granulating agent to a specific medicinal should be ascertained by experimentation with the medicinal in question. The agents discussed here have proven themselves suitable for sodium bicarbonate as representative of a soluble medicinal and calcium gluconate as representative of a slightly soluble medicinal.

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A Note on the Alkaloids of *Rauwolfia micrantha* Hook. f.*

By D. S. RAO and S. B. RAO

IN VIEW of the current therapeutic interest in Rauwolfia, *R. micrantha*, a commonly growing species of South Travancore was subjected to a detailed systematic analysis. The oleoresin fraction prepared by the method of J. C. Gupta, *et al.* (1) on paper chromatography (circular, with butanol-hydrochloric acid-water (50, 7.5, 17.5) showed the following fluorescent bands: deep yellow, R_f 0.80, greenish-yellow, R_f 0.86, and yellowish-green R_f 0.92.

The oleoresin was further fractionated by adsorption ultra-chromatography on alumina. Valuable information regarding the distribution of the fluorescent bases which was of great use in their fractionation could be gathered by circular paper

* Received October 21, 1954, from the Navaratna Pharmaceutical Laboratories, Research Laboratory, Cochin 2, India.

chromatography of the fractions. In this way we were able to isolate the following bases from the oleoresin fraction:

Base *A*	m. p.....264–266°
Base *B*	m. p.....247–248°
Base C	m. p.....157–159°

Base *A* had the characteristic yellowish fluorescence of reserpine. On circular paper chromatograms run with authentic reserpine (R_f 0.98) it gave only a single band (R_f 0.98). Keller's color reaction was positive, blue changing to greenish-yellow. The m. p. of Base *A* was not depressed by the addition of pure reserpine isolated from *Rauwolfia serpentina* oleoresin.

Base *B* had a greenish-yellow fluorescence (R_f 0.90) which could be easily distinguished from a yellowish-green fluorescent band of Base *A*.

Base *C* also showed a pale greenish-yellow fluorescence (R_f 0.86).

The main alkaloidal fraction prepared by the method of A. Furlenmeier (2) after adsorption ultrachromatography on alumina, yielded several fractions. Paper chromatography indicated the presence of at least four bases. Of these we have been able to get only one in crystalline form. Base *D* m. p. 257° gave a HCl salt, m. p. 278°. This was homogeneous paper chromatographically, and showed a greenish-yellow fluorescent band (R_f 0.92). On running with reserpine, separation could be clearly noted. Base *D*, melting point 257°, did not depress the melting point of an authentic sample of ajmalicine (kindly kept at our disposal by Messrs. Riker Laboratories Inc., Los Angeles California). On running mixed paper chromatograms in butanol-hydrochloric acid-water (50, 7.5, 17.5) only one spot could be noted. Further work bearing on its constitution is in progress.

We have also studied the paper chromatographic pictures of *R. serpentina*, *R. canescens*, *R. baddomei*, and *R. densiflora*. Of these the presence of reserpine could be clearly seen in the first two. Characteristic differences of diagnostic importance have also been noted. In view of the easy paper chromatographic separation and characterization of reserpine, a quantitative method for its estimation on similar lines is being worked out. Details with respect to the above will be published elsewhere.

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A Note on Stability of the B Vitamins in Solutions—Crystalline Vitamin B_{12}*

By THOMAS J. MACEK and BEATE A. FELLER

IN A RECENT REPORT (1) it is concluded that vitamin B_{12} is not stable in B complex solution containing thiamine and niacinamide as constituents. For the most part this conclusion appears to be based upon analysis of solutions exposed to elevated temperatures.

Investigations conducted in this laboratory support these findings in a general way, even though we have not encountered as rapid a rate of destruction at elevated temperatures as reported. On the other hand our data also show that under ordinary storage conditions crystalline vitamin B_{12} is stable in aqueous B vitamin solutions for long periods of time.

In this regard, microbiological assay data on parenteral solutions containing other B vitamins showed not more than 10% loss of vitamin B_{12} at *p*H 3.5 and *p*H 4.5 after storage for one year at room temperature. These solutions maintained at 120° for ninety minutes, however, showed losses of vitamin B_{12} amounting to 55% and 65%, respectively. The solutions contained per cc.: crystalline vitamin B_{12}, 15 μg.; thiamine hydrochloride, 10 mg.; riboflavin, 0.5 mg.; niacinamide, 25 mg.; calcium pantothenate dextrorotatory, 5 mg.; pyridoxine hydrochloride, 5 mg.; benzyl alcohol, 15 mg.; and sodium chloride, 9 mg. Dilute hydrochloric acid was used to adjust *p*H. The solutions were sterilized by filtration through microporous porcelain and were subdivided into flame-sealed ampuls.

Microbiological assays for vitamin B_{12} in a flavored B vitamin-iron elixir at *p*H 4.2 indicated only 5% loss of B_{12} after six months' storage at room temperature and 11% loss after six months at 40°. The composition of the elixir per 4 cc. was: Crystalline vitamin B_{12}, 10 μg.; thiamine hydrochloride, 2 mg.; niacinamide, 15 mg.; pyridoxine hydrochloride, 1 mg.; calcium pantothenate dextrorotatory, 0.64 mg.; ferrous sulfate, 20 mg.; glycerin, 0.8 cc.; alcohol, 0.8 cc.; syrup, 0.8 cc.; cherry and lemon-lime flavors and water *q. s.*

Based on our findings, we conclude that solutions of the B vitamins can be prepared in which crystalline vitamin B_{12} is stable for prolonged periods of time at normal storage temperatures. The large losses of vitamin B_{12} encountered when such solutions are stored at elevated temperatures is not unexpected. A paper showing the effect of thiamine hydrochloride on stability of solutions of vitamin B_{12} is in preparation and will be published soon.

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* Received February 2, 1955, from Merck & Co., Inc., Rahway, N. J.

Book Notices

Behringwerk-Mitteilungen. By BEGRUNDET VON E. v. BEHRING. N. G. Elwert, Universitats- und Verlagsbuchhandlung, Marburg/Lahn, 1954. 127 pp. 16 x 24 cm.

The *Behringwerk* has devoted a special issue of its *Mitteilungen* to the centenary exercises held in honor of its founder, Emil von Behring, and of Paul Ehrlich, the founder of chemotherapy, who created his lifework only a few miles' distance from Behring's laboratory. The book is not limited to historical discussion of the achievements of the two great men honored, but also presents important papers representing present-day original research in the fields of immunization and chemotherapy. The contents and format of the work make it a worthy and dignified tribute to Behring, Ehrlich, and the recent resurgence of German science.

The Kidney. (A Ciba Foundation Symposium). A. A. G. LEWIS and G. E. W. WOLSTENHOLME, Editors. Little, Brown and Company, Boston, 1954. xvi + 333 pp. 14.5 x 21 cm. Price $6.75.

This book is a verbatim account of the proceedings at an international symposium on the kidney, arranged jointly by the Ciba Foundation and the British Renal Association. The full texts of the twenty-two papers presented by outstanding authorities in the field are included along with the discussions that followed each paper. The material covered included structural and functional relationships in the kidney, the regulation of acid-base balance, general problems of electrolyte excretion, and the renal share in the volume control of body fluid. While the symposium is of particular interest to the medical group, those interested in pharmacologic considerations of kidney function and behavior might find it informative.

Kurzes Lehrbuch der Pharmaceutischen Chemie, auch zum Gebrauch fuer Mediziner. (Textbook of Pharmaceutical Chemistry also for the use of physicians.) By K. BODENDORF. 4th rev. ed. Springer Verlag, Berlin, 1954. 482 pp. 18 x 25.5 cm. Price *DM.* 31.50

The new edition of this well-known textbook and compendium combines the plan of former editions—arrangement according to chemical principles and precise fluid presentation—with up-to-dateness by the inclusion of the antihistamine group and by considerable enlargement of the chapters on antibiotics, alkaloids, and sulfonamides. The book, which is designed for the student, will also be found useful by the practicing pharmacist and the physician.

Pharmacology in Medicine. Edited by VICTOR A. DRILL, McGraw-Hill Book Co., Inc., New York, 1954. xii + 1172 + 26 pp. Illustrated. 22 x 28.5 cm. Price $19.50.

This new collaborative textbook is designed for both students and physicians. Its purpose is to present the broader aspects of pharmacology along with other necessary data on the drugs used in the practice of medicine with the idea of providing "the medical student and practitioner with a proper understanding of both the value and limitations of drug therapy."

The book is logically divided into eighteen parts, each representing a topic of major pharmacological interest. These broader divisions contain one to fourteen smaller sections or chapters which are concerned with the various groups of drugs. There are 87 such sections, written by 81 authorities, each of whom is or has been engaged in research pertinent to his particular topic. Their experience in many cases encompasses both laboratory and clinical study. Extensive, up-to-date bibliographies are given at the end of each section.

The drugs are discussed in relation to their chemistry; mechanism of action; effects on organ systems; absorption, fat, and excretion; preparations available and dosages; toxicity; therapeutic uses. Where necessary, factors such as tolerance, addiction, synergism, allergenic phenomena, antagonism, etc. are discussed.

Drugs are listed by both their official and common names in the index, which simplifies its use. Each section has its own numbering of pages and illustrations which provides quick reference. The material is presented in a very concise, easy-to-read fashion. There is no lack of continuity due to the fact that this is a collaborative work.

Many subjects in pharmacology have grown so rapidly in recent years that they now require special chapters of their own. Thus, there are chapters devoted to subjects like "General Anesthesia: Intravenous Agents," "Veratrum Alkaloids," "Insecticides, Rodenticides, and Agricultural Poisons," and others. A special chapter on "Radioactive Elements" provides fundamental information on radioactivity as well as a discussion on the biologic effects of ionizing radiation. Presentation of information of this type seems well warranted in view of the increasing use of radioactivity in research, therapeutics, and the threat of the use of atomic weapons.

The printing and binding are of excellent quality. Illustrations and tables are used extensively and these provide good summaries of the data presented.

Dr. Drill and each of the contributing authors are to be highly complimented for their part in the preparation of this text. It is to be recommended to all who are interested in the theoretical and practical aspects of pharmacology.

Hospital Formulary of Selected Drugs. By DON E. FRANCKE. Hamilton Press, Hamilton, Ill., 1954. xvi + 759 pp. 12.5 x 19 cm. Price $5.

As stated in the preface, "This Hospital Formulary of Selected Drugs" has been prepared as a function of the Pharmacy and Therapeutics Committee of the University of Michigan Hospital. The formulary is designed to serve as a readily accessible reference of helpful information for members of the medical and nursing staffs. Containing more than 775 pages, this newly revised formulary reflects the

active selections by medical practitioners of their armamentarium for the diagnosis and treatment of patients.

Although intended for use in the hospital for which it was written, this formulary would serve the needs of any hospital as a useful guide and reference. In fact, it has been accepted for use in several other institutions where the local pharmacy and therapeutics committees have made provisions for certain additions and deletions to the book in order to make it conform to local practices and needs. In addition to its particular usefulness to hospital pharmacists and pharmacy and therapeutics committees engaged in compiling a formulary, this book could be helpful as a reference tool for physicians, dentists, nurses, and others who have occasion to use general information on drugs.

The formulary contains approximately 500 basic drugs of which 298 are U. S. P., 76 N. F., 52 N. N. R. while the remaining do not fall into any of these categories. (Certain of the drugs included in the U. S. P. or N. F. classification are also in the N. N. R. but they are here included only in the figures given for U. S. P. and N. F. drugs.) The author of the formulary has indicated by means of superscription, e. g. U. S. P. XV and N. F. X, those items which are scheduled for inclusion in the forthcoming revisions of these books.

The table of contents indicates the scope and arrangement of the formulary. There are 24 sections of the book devoted to drugs and these are arranged under the following headings: Antihistamine Drugs; Anti-Infective Agents; Antinauseants and Antiemetics; Autonomic Drugs; Cardiovascular Drugs; Central Nervous System Drugs; Diagnostic Agents; Electrolytic, Caloric and Water Balance; Enzymes; Expectorants and Cough Sedatives; Eye, Ear, Nose, and Throat Preparations; Fever-Producing Agents; Gastrointestinal Drugs; Gold Compounds; Heavy Metal Antagonists; Hematinics; Hormones and Synthetic Substitutes; Local Anesthetics; Oxytocics; Serums, Toxoids, and Vaccines; Skin and Mucous Membrane Preparations; Spasmolytic Agents; Vitamins; and Unclassified Therapeutic Agents.

There are also sections devoted to Prescription Writing, Conversion Tables, Biochemical Tables, and a rather comprehensive chapter on Antidotes and the Treatment of Poisoning. The index is well done and contains 20 pages of entries which have been quite adequately cross-indexed according to official as well as proprietary names. The preface contains rules and policies of the Pharmacy and Therapeutics Committee at the University of Michigan Hospital, and other material which would be of general interest to those in the process of developing a formulary.

Monographs of individual drugs are arranged with the official name to the left and the common trade names to the right. This format gives a conspicuous place to both types of names and makes the book easy to use. In most instances the structural formula and chemical name of the basic drug have been included. The general writeup of each drug includes statements on actions, uses, and side effects. This is followed by separate statements on dosage, and the preparations available.

The *Hospital Formulary of Selected Drugs* is a thorough compilation. In some instances, the monographs might even appear too extensive. However, the careful selections of drugs included as well as the supplemental information and the manner in which the material is presented, offers an exceptionally fine example of an up-to-date hospital formulary. The format and total makeup of the book facilitates its use as a reference.

BOOKS RECEIVED

Cellular Metabolism and Infections. E. RACKER, Editor. Symposium held at the New York Academy of Medicine, March 4 and 5, 1954. Academic Press, Inc., New York, 1954. xi + 196 pp. 16 x 23.5 cm. Price $4.80.

Catalysis. Vol. 2. Edited by PAUL H. EMMETT. Reinhold Publishing Corporation, New York, 1955. vi + 473 pp. 16 × 23.5 cm. Price $12.

Industrial Detergency. Edited by WM. W. NIVEN, JR. Reinhold Publishing Corporation, New York, 1955. Illustrated. 340 pp. 16 x 23.5 cm. Price $8.75.

Rapid Detection of Cations. By GASTON CHARLOT, DENISE BÉZIER, and ROLLAND GAUGUIN. Translated by Ralph E. Oesper. Chemical Publishing Co., Inc., New York, 1954. 92 pp. 13.5 x 20 cm. Price $3.

Small-Scale Inorganic Qualitative Analysis. By J. T. STOCK and J. HEATH. Chemical Publishing Co., Inc., New York, 1954. 96 pp. 12.5 x 18.7 cm. Price $2.50.

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Outlines of Enzyme Chemistry. J. B. NEILANDS and PAUL K. STUMPF. John Wiley & Sons, Inc., New York, 1955. viii + 315 pp. 16 x 23.5 cm. Price $6.50.

Drugs in Current Use 1955. Edited by WALTER MODELL. Springer Publishing Company, Inc., New York, 1955. ix + 147 pp. 14 x 21 cm. Price $2.00.

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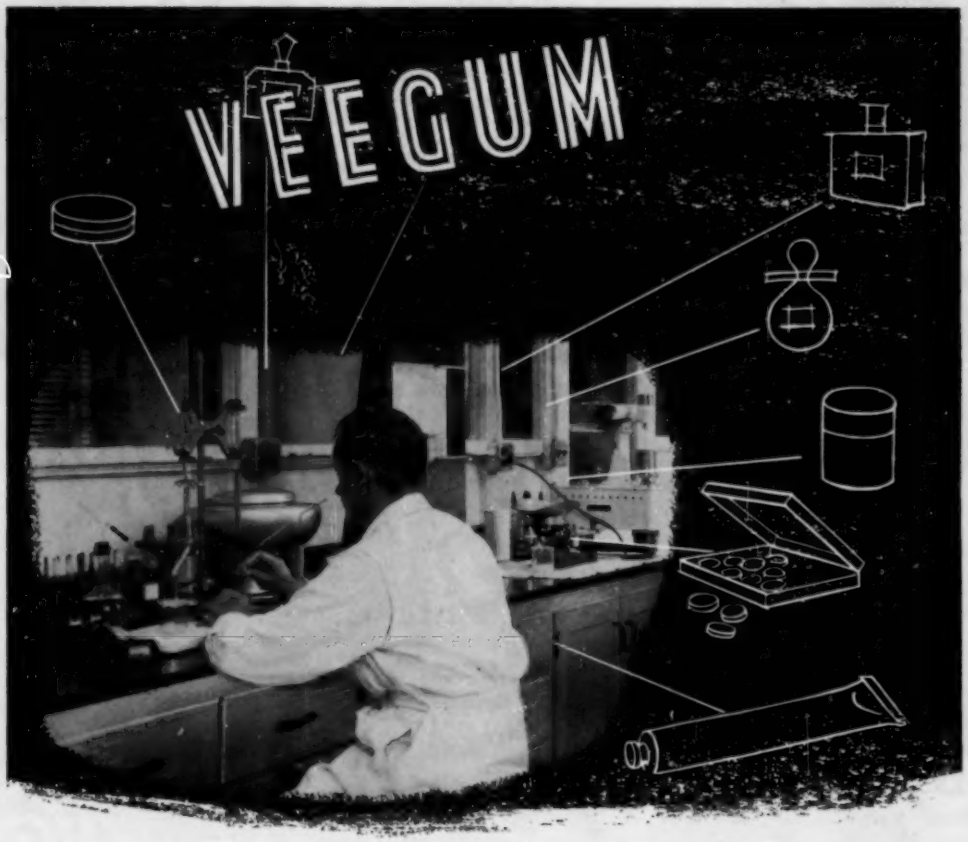

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